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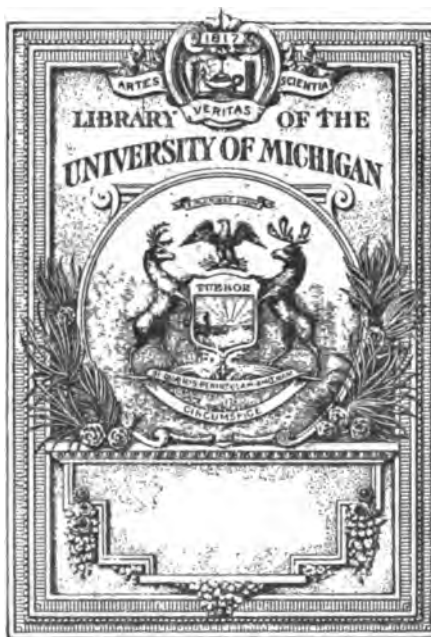
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FIRST SERIES VOLUME X

CHICAGO  
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## **THE PRODUCTION OF MUSCULAR TWITCHINGS**



# ON THE PRODUCTION AND SUPPRESSION OF MUSCULAR TWITCHINGS AND HYPERSENSITIVENESS OF THE SKIN BY ELECTROLYTES

JACQUES LOEB

It has been shown in former publications that a slight variation in the proportion and character of the electrolytes in a tissue is capable of imparting to that tissue properties which it does not possess ordinarily, and it has been suggested that this fact might help us in recognizing the nature of a number of nervous and muscular diseases, and also possibly furnish a means of curing or mitigating them.<sup>1</sup> This paper contains some further contributions to the same subject. It deals with the determination of electrolytes which are liable to produce and inhibit hyperactivity of muscles and hypersensitiveness of the nerves of the skin; and tries to answer the question whether or not the stimulating and inhibiting effects of ions are a function of their valency and electrical charge.

## I. THE PRODUCTION AND SUPPRESSION OF MUSCULAR TWITCHINGS BY ELECTROLYTES

1. Our muscles do not normally contract or twitch rhythmically, but they do so in certain diseases. The main electrolyte in our blood is sodium chloride. When we put a muscle into a pure sodium-chloride solution of the right osmotic pressure (*i. e.*, isotonic with the muscle), the muscle soon begins to twitch rhythmically, and these twitchings may last for several days, or about as long as the muscle lives. But when we add a very small, though definite, amount of a soluble calcium salt, the twitchings will not occur, though the muscle lives longer in such a solution than in a pure sodium-chloride solution. I concluded from this that we owe it to the calcium ions in the blood that our muscles do not twitch or beat rhythmically like our heart.<sup>2</sup>

To test this idea further, Mr. W. E. Garrey and I undertook, in 1899, a series of experiments, not yet published, on the behavior of muscles in solutions of sodium salts whose anions precipitate calcium. The muscle itself contains calcium salts, and we considered it likely that these calcium salts might help in preventing contractions. We therefore thought that by putting the muscle into solutions of sodium salts, which, by entering the muscle, precipitate the calcium contained in it, we might produce still more powerful rhythmical contractions than in a pure sodium-chloride solution. This was found to be true. In solutions of sodium-fluoride, -oxalate, -carbonate, -phosphate, etc., of the proper concentration (1 gram-molecule in 8 liters of the solution), we obtained similar, but more powerful, rhythmical contractions than in sodium-chloride solutions of the same osmotic pressure. Another series of observations con-

<sup>1</sup> J. LOEB, *American Journal of Physiology*, Vol. III (1900), p. 327; Vol. V (1901), p. 362; Vol. VI (1902), p. 411. *Pflüger's Archiv*, Vol. LXXXVIII (1901), p. 68.

<sup>2</sup> *Festschrift für Fick*, 1899. See also S. RINGER, *Journal of Physiology*, Vol. VII (1886), p. 291. In this paper Ringer also mentions briefly the fact that Ba differs in its action from Ca and Sr.

firms the idea that it is due to the calcium salts in our body that our muscles do not show any rhythmical contractions or twitchings. When we inject into the body of an animal any salts that are liable to precipitate calcium, we notice almost immediately twitchings of all the muscles.<sup>3</sup> It seems, therefore, rational that in the pathology of muscular twitchings the concentration of the calcium ions in the blood should be taken into consideration. It is quite possible that abnormal conditions may arise in the body which lead to an increase of such acids in the circulation as diminish the amount of calcium ions in the body, *e. g.*, oxalic acid, or others. The necessary outcome would be muscular twitchings. In that case the administration of calcium salts might cure the disease.

2. In a recent paper I have shown that the antitoxic effects calcium produces when added to a pure NaCl solution are a function of its valency and the sign of its charge, inasmuch as similar effects can be produced by other bivalent or trivalent kations (*e. g.*, Mg, Sr, Ba, Zn, Fe, Co, Pb, Al, Cr), but not by bivalent or trivalent anions.<sup>4</sup> The question arises whether or not the inhibiting effects of Ca ions in the case of rhythmical contractions of muscles are also a function of the valency and electrical charge of the Ca ion. My earlier experiments were not opposed to such a conclusion. I had found that in  $m/8$  solutions<sup>5</sup> of LiCl, NaCl, RbCl, and CsCl rhythmical contractions occur, while small amounts of the chlorides of Ca, Mg, Sr inhibit these contractions. I have since continued these experiments, with the following results: When we put a muscle (the gastrocnemius of the frog was used in these experiments) into a  $m/8$  sodium-acetate solution, the twitchings of the muscle begin at once. The addition of from 3 to 4 c.c. of a  $m$  CaCl<sub>2</sub> solution to 100 c.c. of a  $m/8$  sodium-acetate solution absolutely suppresses all twitchings. But even half the amount suffices for practical purposes, inasmuch as in this case only a few beats occur at the beginning. MgCl<sub>2</sub> and SrCl<sub>2</sub> act like CaCl<sub>2</sub>. But BaCl<sub>2</sub> acts altogether differently. An addition of 5 c.c. of a  $m$  solution of BaCl<sub>2</sub> to 100 c.c.  $m/8$  sodium-acetate solution not only does not stop the rhythmical contractions, but makes them more powerful. Instead of the rapid and rather weak fibrillary twitchings which occur in a  $m/8$  sodium-acetate solution, more tetanic and energetic contractions occur when BaCl<sub>2</sub> is added. I then tried whether the muscle is able to beat in a pure BaCl<sub>2</sub> solution. It goes without saying that in pure solutions of MgCl<sub>2</sub>, CaCl<sub>2</sub>, and SrCl<sub>2</sub> a muscle does not show any rhythmical contraction. In a  $m/8$  BaCl<sub>2</sub> solution, however, the muscle beat for forty minutes; in a  $m/16$  solution, for one and a half hours; in a  $m/32$  BaCl<sub>2</sub> solution, for over an hour; and in a  $m/64$  BaCl<sub>2</sub> solution, for about half an hour. The beats showed the same tetanic form characteristic of the presence of barium. The fact that the beats stop sooner in a  $m/8$  BaCl<sub>2</sub> solution than in a  $m/16$  solution is due to the poisonous effects of barium. The fact that the beats stop also very soon in a

<sup>3</sup> FRIEDENTHAL, *Engelmann's Archiv*, 1901, p. 145.

<sup>5</sup> By a  $m/8$  solution is meant a solution which contains 1 gram-molecule in 8 liters.

<sup>4</sup> *Pflüger's Archiv*, Vol. LXXXVIII (1901), p. 68; *American Journal of Physiology*, Vol. VI (1902), p. 411.

$m/64$   $\text{BaCl}_2$  solution is due to the enormous absorption of water which occurs in such weak solutions.<sup>6</sup>

Similar facts were found for  $\text{Ba}(\text{NO}_3)_2$  and  $\text{Ba}(\text{HO})_2$ . The most striking fact is that the stimulating power of Ba salts is greater than that of the corresponding Na salts. In a  $m/64$   $\text{BaCl}_2$  solution the muscle may begin to beat in a few minutes and may continue to do so for half an hour. I have even occasionally noticed rhythmical contractions in a  $m/128$   $\text{BaCl}_2$  solution. But I have never noticed any rhythmical contractions of muscle in a  $m/32$   $\text{NaCl}$  solution, and as a rule even in a  $m/16$   $\text{NaCl}$  solution the twitchings begin only after a long latent period and last but a short time.

Barium is, however, not the only bivalent kation whose chloride possesses a higher stimulating power than that of the chloride of univalent kations. It was found that the chlorides of the heavy metals are also capable of producing rhythmical twitchings or beats of muscles in much lower concentrations than those found effective in the case of  $\text{NaCl}$  or  $\text{LiCl}$ . In  $m/100$  solutions of  $\text{ZnCl}_2$  strong beats occurred, which, however, did not last long on account of the rapid imbibition of the muscle with water, as well as on account of the poisonous effects of Zn. In stronger solutions than  $m/64$  no beats occurred. The same was true for  $\text{ZnSO}_4$ . Solutions of  $\text{CdCl}_2$  and  $\text{Pb}(\text{NO}_3)_2$  also gave rise to a few contractions in the concentration of about  $m/128$  to  $m/150$ .

The fact that the more concentrated solutions of the salts of heavy metals did not act is probably due to their poisonous effect. It is, therefore, evident that there are a number of chlorides with bivalent kations which are able to produce rhythmical contractions at a lower concentration than  $\text{NaCl}$ . It would, therefore, be wrong to ascribe the inhibiting effect of Ca salts upon rhythmical contractions to the double valency and the positive charge of the Ca-ions.

3. Does the effectiveness of salts for the production of rhythmical muscular contractions increase with the valency of the anion? This is decidedly not the case, as the following table shows. In this table are given the minimal concentrations of the solutions of various sodium salts in which rhythmical contractions occur:

	Salt	Minimal Effective Concentration
Univalent anions	$\text{NaCl}$	$m/16$
	$\text{NaBr}$	$m/16 - m/32$
	$\text{NaJ}$	$m/32$
	$\text{Na acetate}$	$m/32 - m/64$
	$\text{NaF}$	$m/64 - m/96$
	$\text{Na formiate}$	$m/80 - m/160$
Bivalent anions	$\text{Na}_2 \text{ succinate}$	$m/16$
	$\text{Na}_2 \text{SO}_4$	$m/32$
	$\text{NaHCO}_3$	$m/16 - m/32$
	$\text{Na}_2 \text{ oxalate}$	$m/260 - m/300$
Trivalent anions	$\text{Na}_2 \text{HPO}_4$	$m/128 - m/256$
	$\text{Na}_2 \text{ citrate}$	$m/200$

<sup>6</sup> The beats in  $\text{BaCl}_2$  solution often do not begin at once, but after a latent period of from two to fifteen minutes.



It is obvious that the power of favoring rhythmical contractions in muscles is not an unequivocal function of the valency of the anion. It is likewise obvious that the sodium salts whose anions precipitate calcium powerfully, like sodium fluoride, sodium oxalate, and sodium phosphate, are among the most favorable salts to produce rhythmical twitchings. Sodium citrate does not precipitate calcium in the tissues, but prevents its precipitating other compounds, and therefore practically makes calcium inactive.<sup>7</sup> But that the precipitation and inactivation of calcium is perhaps not the only factor involved is shown by the efficiency of sodium formiate. It may be, however, that sodium formiate undergoes further changes in the tissues, and that one of the products formed acts upon calcium.

All these facts suggest that it might be worth while to test the idea whether or not the pathological cases of *muscular* hyperactivity and twitching are due to a lack of calcium in the muscles (or blood), and whether the evil can be mitigated by giving calcium salts to such patients. Experiments must be made in animals to find out whether or not such a treatment would do any harm before any therapeutical experiments in patients should be attempted. It is our intention to take up these experiments in the laboratory.

## II. THE DIFFERENT EFFECTS OF CALCIUM IN THE CASE OF MYOGENIC AND NEUROGENIC RHYTHMICAL CONTRACTIONS

1. While it seems easy to suppress, by the addition of Ca, Sr, and Mg, rhythmical twitchings which originate in the muscle itself, the question arises whether the same means allow us to suppress, with equal ease, muscular twitchings which originate through the central nervous system. The simplest organism in which this can be tested is probably the jelly-fish. These animals contract rhythmically. Their central nervous system is contained in the margin of the swimming-bell, while the center of the animal is said to contain no nerve-elements except scattered neurons. By a cut parallel to the edge we can divide the animal into a marginal part, which contains the central nervous system, and a central part, without a central nervous system.

When this operation is performed, the margin will go on beating in sea-water, while the center will not beat. Romanes, who was (as I believe) the first to make this experiment, drew the conclusion that the central nervous system was the originator of the automatic contractions of this animal. From previous experiments of Aubert,<sup>8</sup> Howell,<sup>9</sup> and Greene<sup>10</sup> on the heart, and my own experiments on the muscles, I concluded that the center of a jelly-fish (*Gonionemus*) did not beat in sea-water on account of the presence of certain ions in sea-water, especially calcium, and I showed that the center of a medusa will beat rhythmically in a pure NaCl or NaBr solution.<sup>11</sup> The center of a medusa whose margin is cut off seems then to behave, to a certain

<sup>7</sup>Sabbatani has shown that, although sodium citrate does not precipitate calcium, it renders it inactive. In the presence of a sufficient quantity of sodium citrate, calcium loses, *c. g.*, its coagulating effect; *Archives Italiennes de Biologie*, Vol. XXXVI (1901), p. 397.

<sup>8</sup>AUBERT, *Pflüger's Archiv*, Vol. XXIV (1881), p. 361.

<sup>9</sup>HOWELL, *American Journal of Physiology*, Vol. II (1898), p. 47.

<sup>10</sup>GREENE, *ibid.*, p. 82.

<sup>11</sup>LOEB, *ibid.*, Vol. III (1900), p. 383.

extent, like the striped muscle. It was of some importance to find out how far this analogy goes. The following six solutions were prepared:

1. 100 c.c.  $m/2$  NaCl
2. 100 c.c.  $m/2$  NaCl +  $\frac{1}{2}$  c.c.  $\frac{1}{8} m$   $\text{Ca}(\text{NO}_3)_2$
3. 100 c.c.  $m/2$  NaCl + 1 c.c.  $\frac{1}{8} m$   $\text{Ca}(\text{NO}_3)_2$
4. 100 c.c.  $m/2$  NaCl + 2 c.c.  $\frac{1}{8} m$   $\text{Ca}(\text{NO}_3)_2$
5. 100 c.c.  $m/2$  NaCl + 4 c.c.  $\frac{1}{8} m$   $\text{Ca}(\text{NO}_3)_2$
6. 100 c.c.  $m/2$  NaCl + 8 c.c.  $\frac{1}{8} m$   $\text{Ca}(\text{NO}_3)_2$

In solution 1 the center of a medusa begins at once to contract very rapidly. The velocity of contractions steadily increases, and very soon it becomes impossible to count the contractions. Occasionally the same happens in solution 2. But in the solutions 3 to 6 the center at first remains perfectly quiet. After a latent period of about ten minutes, often, but not always, contractions begin in solutions 3 to 5, or even in 6, which last sometimes as long as fifteen minutes. These contractions are not as rapid as those observed in a pure NaCl solution, and resemble more the normal contractions of a medusa in sea-water. A series of experiments was undertaken to find out the minimal amount of Ca required to prevent completely all contractions in a pure NaCl solution. In a mixture of 100 c.c. of a  $m/2$  NaCl solution + 3 c.c. of a  $\frac{5}{4} m$   $\text{Ca}(\text{NO}_3)_2$  solution no contractions occurred. A series of experiments with a slightly greater amount of  $\text{CaCl}_2$  were made with the same result.

The same inhibitory effect can be produced if, instead of Ca, Sr or Mg is used. But Ba behaves altogether differently. The following solutions were tested:

- 100 c.c.  $m/2$   $\text{NaNO}_3$ ,
- 100 c.c.  $m/2$   $\text{NaNO}_3$  + 1 c.c.  $m$   $\text{BaCl}_2$ ,
- 100 c.c.  $m/2$   $\text{NaNO}_3$  + 2 c.c.  $m$   $\text{BaCl}_2$ ,
- 100 c.c.  $m/2$   $\text{NaNO}_3$  + 4 c.c.  $m$   $\text{BaCl}_2$ ,
- 100 c.c.  $m/2$   $\text{NaNO}_3$  + 8 c.c.  $m$   $\text{BaCl}_2$ ,
- 100 c.c.  $m/2$   $\text{NaNO}_3$  + 16 c.c.  $m$   $\text{BaCl}_2$ ,

When the center of a medusa was thrown into any of these solutions, the rhythmical contractions began at once. The center behaved as if the Ba ion had not been present, with this difference, however, that the solutions with a larger amount of barium were more poisonous than a pure NaCl solution. *Ba has, therefore, little or no inhibitory effect upon the center of a medusa.*<sup>12</sup>

The analogy between the effect of ions upon muscle and the center of a medusa goes still farther. I pointed out that possibly the Ca ions in the sea-water and the tissues of the medusa prevent the isolated center from beating in sea-water in the same way as the presence of Ca in the blood seems to prevent our muscles from beating. In order to test this idea, I added to the sea-water various salts which precipitate Ca, e. g., NaF and  $\text{Na}_2\text{HPO}_4$ . I found that when a little more of these salts had

<sup>12</sup>Since this was written I have received, through the kindness of Professor Sabbatani in Cagliari, a paper published by his assistant, Dr. Regoli, in which the latter shows that, while Ca and Sr diminish the irritability of

the cerebral cortex, Ba has the opposite effect; REGOLI, "Azione dei metalli alcalino-terrosi sulla eccitabilità elettrica della corteccia cerebrale," *Bollettino d. Societate tra i Cultori delle Scienze etc. in Cagliari*, Torino, 1901.

been added than required to precipitate all the Ca in the sea-water, the center behaved indeed in the same way as if it had been put into a pure NaCl solution. When a little less  $\text{Na}_2\text{HPO}_4$  was added, the beats began after a latent period, which varied according to the amount of  $\text{Na}_2\text{HPO}_4$  added. Rapid contractions began at once when 32 c.c. of a  $m/8$   $\text{Na}_2\text{HPO}_4$  solution was added to 68 c.c. of sea-water. The same result was obtained when 16 c.c. of a normal NaF solution was added to 100 c.c. of sea-water.

The addition of about 13 c.c. of  $m$  sodium-citrate solution to 100 c.c. of sea-water also brought about immediate contraction of the isolated center. This salt does not bring about a precipitation of Ca in the sea-water or the tissues, but excludes the action of Ca ions in another way.

I did not succeed in bringing about such results with the addition of  $\text{Na}_2\text{SO}_4$  to sea-water. Even the addition of 32 c.c. of  $m$   $\text{Na}_2\text{SO}_4$  to 100 c.c. of sea-water did not give rise to contractions, although the irritability of the center was increased. Experiments with the addition of  $\text{NaHCO}_3$  remained also negative. But as only a few experiments were made with  $\text{Na}_2\text{SO}_4$  and  $\text{NaCHO}_3$ , it is possible that a continuation of the work might lead to positive results.

It is, therefore, obvious that the centers can be caused to beat through a diminution of the amount of Ca they contain, and it may be further argued that the presence of Ca in the sea-water is the cause, or at least one of the causes, that prevent the centers from beating in sea-water.

It should, however, be added that, while a certain diminution of Ca in the center is necessary for the development of rhythmical contractions, the diminution has its limit. It appears that, if too much Ca is removed from the tissues, the beats will also cease. This is demonstrated by the following facts: When we put the center of a medusa into sea-water to which enough sodium citrate has been added, beats begin at once, last for a certain time, and then cease. If at this time the centers are put back into sea-water with less or no sodium citrate, beats will begin again. The explanation of this phenomenon seems to be as follows: The normal center of a medusa contains too much Ca for spontaneous rhythmical contractions. If we put a center into sea-water to which a large amount of NaF,  $\text{Na}_2\text{HPO}_4$ , or sodium citrate has been added, so much of the salt will diffuse at once into the organism that at least in the superficial cells enough Ca will be eliminated from the field of action to allow the spontaneous contractions to begin. Subsequently the same will happen in the deeper cells. The process of elimination of calcium in the cells proceeds, and very soon a period comes when the loss of Ca in all the cells will be too great for the contractions to go on. If, as soon as this occurs, the center is thrown into normal sea-water, or sea-water with only a little sodium citrate or phosphate, citrate and phosphate anions will diffuse back from the tissues into the sea-water, or Ca ions will diffuse into the cells, or both phenomena will occur, and beats will again begin. The same reasoning applies probably to the rhythmical contractions of muscles and the apex of the heart.

2. When we put the margin containing the central nervous system into a pure NaCl solution, it behaves very much like the center, *e. g.*, it begins to beat very rapidly, and the rapidity of the beats increases, at first steadily, until the poisonous effects of the pure NaCl solutions make themselves felt. But even the addition of large quantities of Ca does not inhibit these contractions. For instance, when we add from 2 to 5 c.c. of a  $\frac{5}{4}m$  solution of  $\text{Ca}(\text{NO}_3)_2$  to 100 c.c. of  $m/2$  NaCl solution, the margin at once begins its rapid beats. The only effect the addition of calcium has is to make the rate of the beats a little slower than without calcium. I thought at first that the stimulus of the wound caused by the cutting off of the margin might be responsible for these contractions in the presence of calcium. But this is not the case, for if we put a whole *Gonionemus* intact into any of these solutions, it behaves like the isolated margin. The only possible inference is that the margin is much more immune toward the inhibiting effects of calcium than the center, a fact which I have pointed out already in a former paper.<sup>13</sup> In a pure  $\text{CaCl}_2$  solution the margin will not beat.

Inasmuch as the essential difference between center and margin which accounts for this difference in the effect of calcium is the presence of the central nervous system in the margin, it may follow from these observations that for the suppression of twitchings of a nervous origin larger doses of calcium might be required than for the suppression of twitchings of muscular origin. Preliminary experiments on the motor nerves of frogs seem to harmonize with this idea. This suggests the possibility that, while a calcium treatment might be advisable for the cure of myogenic twitchings, for the suppression of neurogenic twitchings so much calcium might be required as to exclude the use of this remedy. This, too, is a point which further experiments on animals must decide before the matter may be tried in patients.

### III. THE PRODUCTION OF HYPERSENSITIVENESS OF THE SKIN BY ELECTROLYTES

1. In a former paper I have shown that, aside from the rhythmical twitchings, the salts whose anions precipitate or inactivate calcium also make muscles and motor nerves sensitive to stimuli which normally would not affect these organs. For example, when we put a fresh muscle for one or more minutes into a  $m/8$  solution of sodium citrate, a peculiar form of irritability arises (contact irritability).<sup>14</sup> Whenever the muscle is taken out of the solution it goes into powerful tetanic contractions, which cease at once and give way to relaxation of the muscle as soon as the latter is put back into the solution. When this hypersensitive condition is once established, the contractions can be produced whenever the muscle is changed from any aqueous solution to any other non-aqueous medium, while the contractions cease when the muscle is put back into an aqueous medium, no matter whether the latter be a solution of an electrolyte or a non-conductor. It is rather striking that these phenomena do not occur when the above-mentioned solutions call forth at once the rhythmical contractions

<sup>13</sup> *American Journal of Physiology*, Vol. III (1900), p. 383.

<sup>14</sup> *Ibid.*, Vol. V (1901), p. 362.

mentioned in the previous part of this paper. It almost looks as if there existed two allotropic states of the muscle substance, the one giving rise to rhythmical twitchings, the other to the peculiar tetanic contractions (contact reactions) just referred to.<sup>15</sup> Ultimately, however, in all cases rhythmical twitchings are produced.

As far as motor nerves are concerned, I have shown in the same paper that the same salts which produce this contact reaction produce hypersensitiveness of the nerve and ultimately rhythmical contractions of the muscle when acting upon the nerve alone.

It might be mentioned here in parenthesis that these facts may throw some light upon the action of cathartics. All the salts which give rise to the above-mentioned contact reaction or hypersensitiveness act as cathartics when introduced into the intestine. The common explanation of their action is the one which, I believe, was first suggested by Schmiedeberg, namely, that these salts prevent the absorption of liquids from the intestine, and that this retention of liquids causes the cathartic effect. I will not deny the effect of these salts upon the phenomena of absorption of water from the intestine,<sup>16</sup> but it is obvious from our experiments that the same salts must increase the irritability of the nerves and muscles of the intestine, and that this must facilitate the production of peristaltic motions, possibly through the mechanical or contact stimuli of the fæces upon the nerve-endings or the muscular wall of the intestine.

2. These experiments suggested the idea whether or not electrolytes are capable of producing also a hypersensitiveness of the skin and conditions that may be comparable to the conditions of hyperæsthesia or hyperalgesia. It is well known that when we suspend a pithed frog vertically so that its legs hang down, the latter will be lifted at once when they are dipped into an acid or alkali of a certain concentration, while no such reaction occurs when they are dipped into water. The reaction of the animal to acid may be so violent as to suggest to a layman the idea that it is suffering intense pain. I wondered whether by an alteration of the nature and proportion of ions in the skin the sensitiveness could be increased or varied in such a way as to make the skin as sensitive to the contact with pure water as it naturally is to strong acid. The experiments resulted in my finding certain solutions of electrolytes which did not seem to affect the animal directly, but yet made it extremely sensitive toward contact with water. The best solutions for this purpose are, as far as my present experiments go,  $\text{AlCl}_3$  and sodium-citrate solutions. The way of proceeding is as follows: A number of solutions, say of  $\text{AlCl}_3$ , are prepared, namely,  $m/32$ ,  $m/16$ ,  $m/8$ ,  $m/4$ , and possibly  $m/2$ . Then the weakest of the solutions is first brought in contact with the feet of the frog. If the feet are not withdrawn, the next stronger solution is used, and, if no reaction occurs, the next stronger. If one thus succeeds in keeping the feet of the animal for one minute or more in the  $\text{AlCl}_3$  solu-

<sup>15</sup> This difference is emphasized by the fact, found by my pupil, Dr. Zoethout, that an addition of potassium favors the contact reaction. As far as rhythmical contractions are concerned, K has an inhibiting effect.

<sup>16</sup> CUSHNET AND WALLACE, *American Journal of Physiology*, Vol. I (1899).

tion, subsequent contact of the feet with common tap-water or distilled water makes the animal act as if the water caused the most excruciating pain. The feet are violently withdrawn, rubbed against each other in a way that one notices otherwise only when the feet are dipped into strong acids. If the  $\text{AlCl}_3$  solution chosen is too strong, the animal will not leave its feet in the solution, but will try to withdraw them. But in that case its attempts at withdrawing its feet from the solution are never as violent as the subsequent attempts at withdrawing the feet when brought into contact with common water. The stronger the solution of  $\text{AlCl}_3$  is in which the feet had been kept, and the longer they had been in the solution, the stronger their sensitiveness toward water will become.

Sodium citrate acts very similarly to aluminium chloride. As the latter is slightly acid and sodium citrate slightly alkaline, the possibility was suggested that the  $\text{H}$  and  $\text{HO}$  ions are responsible for the hypersensitiveness. While it is possible to produce occasionally a slight hypersensitiveness toward common water by a pure solution of  $\text{NaOH}$  or  $\text{HCl}$ , the results are very unreliable. It is practically the same if one tries to use  $\text{NaCl}$  solutions to which slight and varying quantities of  $\text{HCl}$  or  $\text{NaOH}$  have been added. Better results can be obtained with solutions of oxalates, sulphates, carbonates, and phosphates. The sodium salts are preferable to the potassium salts, for the animal withdraws its feet much more rapidly from the solution of a potassium salt than from the solution of the corresponding sodium salt. This makes it difficult in the case of potassium salts to saturate the foot with the sufficient number of ions to induce the hypersensitiveness.

It goes without saying that the hypersensitiveness which can be produced by  $\text{AlCl}_3$  and sodium citrate does not make itself felt toward water alone, but to salt solutions also. One can find a minimal concentration for each solution of an electrolyte at which a pithed frog almost instantly withdraws its feet when they come in contact with the solution. This minimal concentration is considerably lowered after a treatment of the foot with an  $\text{AlCl}_3$  or sodium-citrate solution.

The production of hypersensitiveness is only one side of the problem. The mitigation of the hypersensitiveness is the other side. The violent reactions of a frog when its feet are dipped in tap-water after a treatment with  $\text{AlCl}_3$  can be stopped instantly when the feet are put into a normal solution of cane sugar. When weaker solutions of cane sugar are used the feet are withdrawn, and the attempts at withdrawing become the more noticeable and violent the weaker the sugar solution is. Very concentrated solutions of urea, *e. g.*, 2*n* solutions, act similarly, but not so powerfully as cane sugar. Glycerine solutions gave no such results; neither have I been able to find as yet any solution of an electrolyte which acted this way. The fact that only very concentrated solutions of cane sugar or urea inhibited the hypersensitiveness gave rise to the idea that the diffusion of water out of the foot might be the inhibiting factor, and that a stream of diffusion in the opposite direction, namely, from the outside into the skin, might give rise to a withdrawal of the foot. The latter idea

could be tested. When the feet of a pithed frog are dipped into a normal solution of cane sugar, they are not withdrawn, no matter how long they remain in the solution. But if subsequently (after several minutes) the feet are put into pure water, after a few (five to ten) seconds the feet are energetically withdrawn. In this case, obviously water diffuses into the skin, which previously had lost water.

There may be electrolytes which act similarly to cane sugar, but I have not yet found them. Every solution of an electrolyte causes, above a certain concentration, an immediate withdrawal of the feet, and this withdrawal is the more energetic the more concentrated the solution. This differs from the behavior of sugar and urea, which above a certain concentration have the opposite effect.

The lowest concentration at which the solutions of various electrolytes will cause a pithed frog to withdraw its feet instantly or in from five to ten seconds, is about as follows:

HCl, $m/240$ or less	CaCl <sub>2</sub>	} $m/8$ or a little less
NaOH, $m/80$ or less	SrCl <sub>2</sub>	
AgNO <sub>3</sub> , $m/180$ or less	BaCl <sub>2</sub>	
FeCl <sub>3</sub> , $m/60$ or less	MgCl <sub>2</sub>	
CdCl <sub>2</sub> } $m/32$ to $m/16$	KCl, $m/8$ to $m/4$	} $3m/8$ to $m/2$
HgCl <sub>2</sub> }	NH <sub>4</sub> Cl, $m/4$ to $3/8m$	
AlCl <sub>3</sub> , $m/16$ to $m/8$	NaCl	
	LiCl	

It almost looks as if the coagulating effect of the kations upon proteids was of some importance. The powerful effects of Ag, Cd, and Hg interfere somewhat with the conclusion that we are dealing with a pure valency effect, which otherwise seems to make itself felt. If, instead of the chlorides, the nitrates or sulphates of the same metals are chosen, the order of efficiency seems to remain practically the same, as far as can be judged from an as yet incomplete series of experiments.

As far as the anions are concerned, the order of efficiency is for the sodium salts about as follows:

Na <sub>2</sub> oxalate, $m/8$	NaHCO <sub>3</sub>	} $3/8m$ to $m/2$
Na <sub>2</sub> citrate, $m/8$	Na formiate	
Na <sub>2</sub> SO <sub>4</sub> , $m/4$	Na <sub>2</sub> succinate	
NaHPO <sub>4</sub> , $m/4$	NaCl	
NaF, $m/4$ to $m/2$		

In this case, as in the case of rhythmical contractions, the oxalates and citrates are the most powerful anions of this series. It is clear that, in the determination of the lowest concentration of a salt which is still able to cause the immediate withdrawal of the foot, one must remember that a number of solutions (*e. g.*, AgNO<sub>3</sub>, AlCl<sub>3</sub>, FeCl<sub>3</sub>, HCl, NaOH, Na<sub>2</sub> citrate, etc.) have an after-effect which makes itself felt in an increase of irritability. Other solutions (*e. g.*, those of calcium salts) may possibly have the opposite effect, namely, to raise the threshold of stimulation for subsequent tests.

It was of some interest to ascertain whether the results in these experiments were produced through an action of the electrolytes upon the nerve-endings, or upon the nerves themselves. In experiments on frogs whose skin had been removed from the feet, the results described in this paper could not be produced. The experiment of putting the nerves themselves into the above-mentioned solutions remained practically without effect. It is possible that with solutions of much greater concentration results may be obtained. It is, therefore, certain that the results observed in our experiments are due to an action of the electrolytes upon the nerve-endings in the skin, and not to an action upon the sensory nerves.<sup>17</sup>

#### IV. CONCLUSIONS

The experiments mentioned in this paper were undertaken with two aims in view, a practical and a theoretical one. As far as the former is concerned, it follows from our investigations that abnormal muscular twitchings and contractions may be brought about in an organism by a reduction in the proportion of calcium (or magnesium) in the muscles or the blood, or an increase in the proportion of Na and other kations. In view of the fact that thus far no explanation has been found for pathological phenomena of this kind, it becomes of some importance to see whether or not in certain of these diseases the relative amount of calcium ions in the blood is diminished. If this should be the case, the administration of calcium would be the cure for these diseases, which thus far have been beyond medical control. It is also apparent from our experiments that for the suppression of neurogenic twitchings or contractions more calcium may possibly be required than for the suppression of myogenic twitchings. There has thus far been no clue as to the origin of hypersensitiveness or hyperalgesia of the skin. Our experiments show that slight variations in the proportion of certain ions in the skin can cause an enormous hypersensitiveness.

As far as the theoretical side of the paper is concerned, it was our aim to test the idea whether or not the "stimulating" and inhibiting effects of ions are an unequivocal function of their electrical charge or valency. Over a year ago I tested the same idea without being able to obtain positive results, and nothing was said about the subject in the paper in which the results were published.<sup>18</sup> The test was continued in the above-mentioned experiments, with results which, in my opinion, are equally questionable, if not altogether negative.

<sup>17</sup> The chemical irritability of muscles is, as far as electrolytes are concerned, also greater than that of motor nerves. The reverse is true for electrical stimulation.

<sup>18</sup> *American Journal of Physiology*, Vol. VI (1901), p. 362.





**WEIGHT OF THE CENTRAL NERVOUS SYSTEM  
OF THE FROG**



# ON A FORMULA FOR DETERMINING THE WEIGHT OF THE CENTRAL NERVOUS SYSTEM OF THE FROG FROM THE WEIGHT AND LENGTH OF ITS ENTIRE BODY

HENRY H. DONALDSON

AS THE living substance which constitutes the animal body becomes differentiated into distinct tissues, the animal as a whole becomes more highly organized. The fundamental tissues which are thus formed — namely, the epithelial, connective, muscular, and nervous — are distinguished by the fact that each of them exhibits one or more of the general characteristics of protoplasm developed to a greater or less degree, whereas other of the characteristics are much less evident or apparently entirely lacking. The combined activities of these differentiated tissues are exhibited in the life-history of the entire animal.

For the understanding of such an animal it is important to know the proportions of the several tissues present in any instance, and whether we study the animal from the standpoint of the number and size of the cell elements which constitute each tissue, or from the more general standpoint of the weight of modified living substance possessing the peculiar physiological characteristics of the tissue, the animal could be described in terms of the analysis, that is, in quantitative terms of the several systems of tissues which compose it.

Thus, when tested in this way, animals like the dog, rabbit, and cat are found to be dissimilar in their make-up, and a snake, for example, has a different tissue composition from a frog. To determine that these animals are thus differently constituted is merely a first step, and is naturally followed by the attempt to determine whether there are any laws governing the quantitative relations of these tissues either in the animal series or in the same animal during its life-cycle.

If it could be shown that one system varies in a definite relation to any or all the others, we should have made a further step toward a comprehensive knowledge of the animal examined; and it is believed that the facts here to be presented constitute such a step.

The following paper describes the weight relations of the central nervous system (brain and spinal cord combined) of the frog, to the tissues constituting the rest of the body. The connection of this investigation with work already done along similar lines can be stated very briefly.

An attempt by Snell (1892) to correlate the increase in the weight of the encephalon with some change in the remainder of the body led him to conclude that among mammals and birds the weight of the encephalon increased in proportion to the area of the body, when animals of different sizes, but otherwise similar, were compared.

DuBois (1898) took up this conclusion of Snell and elaborated it (for mammals alone), making a more careful analysis of the conditions underlying the calculation, and finally giving a formula for the relative weight of the encephalon in mammals, which is very satisfactory, and which differs but slightly from the formula given by Snell.

The study of the work of Snell and DuBois led me to attempt the extension of their results. Their formulæ apply to the *relative* weights of the encephala in mature mammals, so that, in order to get the absolute value, the weight of one of the pair of encephala must be known. They do not discuss the increase of the encephalon in weight in the same mammal during its growing period, nor do they extend their observations to other classes of vertebrates or to the entire central nervous system. In the present instance we have sought to determine whether we could obtain a formula which would express the weight of the entire central nervous system at any time during the growing period of an animal, and in this instance have chosen the frog. For the study of this problem there were available in the laboratory records on two species of frogs, the bullfrog and leopard frog. It may be noted in passing that for a study of this kind the frog presents certain advantageous peculiarities, as it exhibits only a comparatively slight alteration in the bodily proportions during growth.

Like other vertebrates, its increase in length is most rapid during the earlier portion of the growing period, and later, the increase in length becoming slow, the body enlarges at right angles to its long axis, and the animal becomes thicker.

Nevertheless, the weight relations between the muscles of the legs and the remainder of the body remain nearly constant (Donaldson, 1898; Donaldson and Schoemaker, 1900), and in this respect the frog shows but very slight changes in proportion.

In the foregoing characters the two species which have been examined, the bullfrog (*Rana catesbiana*) and the leopard frog (*Rana virescens*), were nearly alike. For each species the series of observations was extensive, comprising data on the body weight and length, and also on the weight and length of the brain and spinal cord, together with other measurements not needed for this investigation (Donaldson, 1898; Donaldson and Schoemaker, 1900).

On looking at the curve previously published, for the weights of the brain and spinal cord arranged according to the body-weight (Donaldson and Schoemaker, 1900, p. 117), it appeared that the weights for the central nervous system (brain and spinal cord combined) were so related as to suggest a logarithmic curve, and this suggestion was at once tested.<sup>1</sup>

The trial was made by forming a curve depending on the logarithms of the body-

<sup>1</sup> In a paper entitled "Zur Anthropologie des Rückenmarkes" (*Corresp.-Bl. d. deutsch. Anthropol. Gesellsch.*, No. 10, 1895), RANKE presents observations on the weight of the spinal cord in dogs of different body-weights. He suggests that the curve which illustrates his Tabelle 3 has the form of a logarithmic curve, but does not test the suggestion. I have determined the curve formed by the logarithms of

the body-weights of the dogs examined, and find that it gives a much flatter curve than that based on the weights of the spinal cords. In this instance, therefore, a logarithmic relationship between the nervous system (spinal cord) and the rest of the body does not appear, but, so far as I am aware, this is the first record of the suggestion that such a relationship might exist.

weights. To raise these logarithms to the value of the observed weights of the central nervous system they required to be multiplied by a constant factor. It was found that the factor which gave a correct value for the smallest frog was too small for all of the succeeding cases, the resulting numbers falling more and more below the observed numbers as the frog became larger. In order, therefore, to make the curve based on the calculated weights fit with that based on the weights observed, there was needed a second factor, the value of which should steadily increase as the body-weights of the frogs became heavier. Such a factor was found in the length of the frog, which increases rather rapidly at first and more slowly later. The unmodified lengths showed, however, too rapid an increase in the course of the series, but various trials revealed that the fourth root of the lengths gave a set of numbers which could be satisfactorily used.

It was found, then, that the number obtained by multiplying the logarithm of the body-weight by the fourth root of the length of the body was always a nearly constant fraction of the observed weight of the central nervous system. In the case of the bullfrog the fraction thus obtained was one-thirtieth of the observed weight, while in the case of the leopard frog it was one twenty-eighth. It could, therefore, be made equal to the observed weight by multiplying it by a constant factor having the value of the denominator of the fraction. In this manner a formula was developed as follows :

$$C.N.S. = (\text{Log } W \times \sqrt[4]{L}) C.$$

Here the weight of the central nervous system (*C.N.S.*), in milligrams, is made equal to the logarithm of *W*, the body-weight, expressed in grams, multiplied by the fourth root of *L*, the length of the body, in millimeters, the product of these factors being raised to the value of the observed weight of the central nervous system by multiplying by a constant, *C*. This constant, in the case of the bullfrog, has the value of 30, and, in the case of the leopard frog, the value of 28. To illustrate the application of this formula, we may take as an example the first record, No. 6, in Table I, p. 7. Here *W*, the weight of the body, is 5.02 grams, and *L*, the length of the body, is 93 millimeters. As this is a bullfrog, the value of the constant *C* is 30. Thus :

$$\begin{aligned} C.N.S. &= (\text{Log } W \times \sqrt[4]{L}) 30 \\ &= (0.7007 \times 3.105) 30 \\ &= (2.17) 30 = 65.1 = 65 \text{ milligrams.} \end{aligned}$$

The calculated weight of the central nervous system is therefore 65 milligrams. The observed value was 62 milligrams; thus the calculated exceeded the observed value by 4.8 per cent. In like manner the weight of the central nervous system was calculated in each of the cases entered in the table. While the formula applies to all the cases which are presented in the tables given in this paper, it does not apply to all the cases in the original tables (Donaldson, 1898, pp. 328-30; Donaldson and Schoemaker, 1900, pp. 120, 121), and its validity can, therefore, be seriously questioned, unless we are able to show that those cases to which it does not apply are capable either of

explanation or correction. The formula is constructed so as to express the normal changes in the weight and length of body as related to the weight of the central nervous system—changes which are taking place as the frog grows larger. But it remains to be determined, first, how early in the history of the frog the formula can be applied, and, second, whether sex, season, and nutritive conditions are able to affect the result; the nutritive condition including not only those changes which may occur from day to day, but those which occur from spring to autumn.

Repeated examination shows that the formula does not apply to frogs until they have attained a body-weight of approximately 5 grams. For example, in a bullfrog with a body-weight of 3.53 grams the observed weight of the central nervous system was 56 milligrams, whereas the calculated weight was only 49 milligrams. A similar result is obtained when the test is applied to the leopard frogs under 5 grams of body-weight; hence, for frogs of this size, the calculated weight of the central nervous system is too small.

The failure of the formula to apply to the smallest frogs is probably due to the precocious enlargement of the central nervous system—a character of all young vertebrates, and one still evident in frogs when less than 5 grams in weight. We conclude, therefore, that the relations found in the mature frogs are not established until they have attained a body-weight above 5 grams. From this point on the formula applies to all normal specimens.

For the consideration of other sources of error it will be most advantageous to examine the two species of frogs separately. We begin with the bullfrogs.

The original table for the bullfrogs (Donaldson, 1898) contains fifty-two cases. The first five cases (Nos. 1–5) are from frogs below 5 grams in body weight, and for this reason are excluded. Among the remaining forty-seven cases, six (Nos. 7, 32, 34, 37, 40, 47 in the original table) are marked “dry,” which means that through drying their body-weight had been reduced below the normal. These also are excluded. No. 9 in this table is plainly abnormal, as is seen by comparing the body-weight with the length (body-weight, 8.75 grams; length, 127 millimeters), and for this reason is also excluded. For the foregoing exclusions no explanation is required, as, under the circumstances, one could not expect the formula to apply to them. There are, however, six more cases to be excluded, namely Nos. 10, 11, 43, because the body weight had been increased by the absorption of water; and Nos. 45, 48, and 49, because long captivity had produced a loss of weight through starvation.

The absorption of water by frogs whose vitality is much reduced is a familiar reaction, and the effects of starvation have been reported in earlier observations from this laboratory (Donaldson and Schoemaker, 1900, p. 112).

In this series no correction for season is required, as the records are all from July and August frogs, and hence comprise midsummer frogs only.

The final table contains, therefore, thirty-four records of approximately normal frogs to which the formula had been applied. These are presented in Table I.

TABLE I

Containing thirty-four records from bullfrogs (based on Table VII, Donaldson, 1898). This table shows in successive columns the original tabular number, the sex, body-weight, length, and the observed and calculated weights of the central nervous system in milligrams. The last two columns show the percentage deviation of the calculated from the observed weights of the central nervous system; the percentages being computed on the observed values as the standard.

TABULAR NUMBER	SEX	BODY		WEIGHT OF CENTRAL NER- VOUS SYSTEM IN MILLIGRAMS		PERCENTAGE DEVIATION OF CALCULATED FROM OBSERVED WEIGHTS	
		Weight in Grams	Length in Millimeters	Observed	Calculated	Deficiency	Excess
6	Male	5.02	93	62	65	...	4.8
8	Male	5.38	95	72	68	5.5	...
12	Male	11.37	125	108	108	1.8	...
13	Female	13.77	136	116	115	0.8	...
14	Female	16.03	145	122	125	...	2.4
15	Male	20.33	159	144	139	3.4	...
16	Male	27.33	167	153	155	...	1.3
17	Female	27.51	173	170	157	7.6	...
18	Male	32.95	184	165	168	...	1.8
19	Female	36.32	182	163	172	...	5.5
20	Female	37.46	188	185	175	5.4	...
21	Male	49.50	192	181	189	...	4.4
22	Female	49.82	202	187	192	...	2.6
23	Female	50.43	203	200	193	3.5	...
24	Male	51.77	200	201	193	3.4	...
25	Female	58.46	210	184	202	...	9.7
26	Female	60.12	211	205	203	1.9	...
27	Female	66.67	218	215	210	2.3	...
28	Female	73.05	231	212	218	...	2.8
29	Male	76.69	231	233	220	5.5	...
30	Male	87.05	231	223	227	...	1.7
31	Male	98.00	240	226	235	...	3.9
33	Male	144.50	280	255	265	...	3.9
35	Male	146.70	284	273	267	1.4	...
36	Female	146.90	275	279	265	5.0	...
38	Female	169.50	275	262	272	...	3.4
39	Male	184.60	284	287	275	4.1	...
41	Male	191.76	313	290	287	...	1.0
42	Male	199.10	312	299	290	3.0	...
44	Male	212.50	304	306	292	4.5	...
46	Female	225.20	303	305	294	3.6	...
50	Male	244.60	313	292	301	...	3.0
51	Female	272.10	340	306	314	...	2.2
52	Male	313.00	343	321	322	...	0.3
Average.....				212	211	3.8	3.0

Difference, 1 milligram; percentage, 0.4.



This table gives the original tabular number, sex, body-weight (without ovaries in the case of the females), length from tip of nose to tip of longest toe, observed and calculated weights of the central nervous system, together with the percentage by which the calculated departs from the observed weight; the observed weight being always considered as the standard. The conditions under which these measurements were made are given in full in an earlier paper (Donaldson, 1898, pp. 323 ff.). In the foregoing table the records in each case are for single observations. When the averages of the weights of the central nervous system, observed and calculated, are determined, it is seen that the average of the observed weights is 212, while that of the calculated is 211, thus giving a difference of only 0.4 per cent. That this small difference is the expression of discrepancies that are only slight is indicated by the fact that, if the entire series of records be divided into three groups, formed respectively by the first eleven, second eleven, and last twelve, and the difference in the average values of the observed and calculated weights be taken for each group, we obtain the percentage differences given in the following table:

TABLE II

To show the average percentage differences in the values of the observed and calculated weights of the central nervous system in three groups, formed from the records in Table I.

GROUP	TABULAR NUMBERS	NUMBER OF RECORDS	PERCENTAGE DIFFERENCE; THE OBSERVED VALUES BEING TAKEN AS THE STANDARD	
			Deficiency	Excess
A	6-21	11	...	1.0
B	22-33	11	0.6	...
C	35-52	12	...	0.8

It is thus seen that the percentage difference between the averages does not in any group exceed 1 per cent., and consequently we may infer that, if the records were based on averages of eleven or more individuals for each entry, the agreement of the observed and calculated values would be well within 1 per cent.

Another method of testing these results is by determining the relation of the percentage differences calculated for the individual cases. On enumerating the cases in which the calculated values are in excess, it is found that they are just seventeen, or one-half the total number of records, thus leaving seventeen cases where the calculated values are below those observed. Table I shows that the average value of the percentage deviations exhibiting deficiency is 3.8 per cent., while for those in excess it is 3.0 per cent. The plus and minus percentage deviations, therefore, nearly balance, as they should do if they depended on accidental causes.

We see, therefore, that the formula gives results very close to those observed. On

looking at the curve (Fig. 1) we note that the calculated weights vary less from frog to frog than do those observed, and it thus happens that the line joining the dots which mark the calculated weights threads its way between the crosses which indicate the observed weights. Thus the weight of the central nervous system as calculated is less irregular than that directly observed.

Before commenting further on these results the observations on *Rana virescens* will be presented. In the case of *Rana virescens* the original table contained thirty-six records (Donaldson and Schoemaker, 1900, p. 120). Of these, Nos. 1, 2, 3 are at once excluded as being below the 5-gram limit. Of the remaining 33, one, No. 16, through some error, has a body-weight too small for its length (27.19 grams body-weight; 195 millimeters length), and four more, Nos. 33, 34, 35, 36, all of them spring frogs, have body weights which are manifestly too small, as is shown by the relation of these records in the curve already presented (Donaldson and Schoemaker, 1900, Chart I, p. 117). As there are no data for correcting these last four records, they are excluded from the series here used. After these removals there remain twenty-eight records taken at different times from April 14 to September 15. Some unpublished work on the seasonal change in the nervous system of the frog shows that in frogs of the same body-weight the weight of the central nervous system is subject to a rhythmic change, thus altering according to the season of the year.

During the past twelve months observations have been carried on in this laboratory with a view to following this change in some detail, and at present we have at hand data which enable us to correct the weight of the central nervous system in these frogs in the early and late season so as to make the observations taken at those times comparable with the records from midsummer frogs. To standardize these early and late records which appear in Table IV, corrections have been made in twelve instances in accordance with a fixed scale. This scale is based on the following observations: It appears that frogs of a given body weight, just after they emerge at the end of March or the first of April, have a relatively small weight of central nervous system.

TABLE III

Showing the corrections made in the spring and autumn frogs, the weight of whose central nervous system appears in Table IV.

No. in Table IV	Date	Percentage Correction	No. in Table IV	Date	Percentage Correction
31	April 14	+4	23	June 5	-5
17	April 19	+4	28	June 5	-5
13	May 21	-1	10	June 9	-5
28	May 21	-1	29	September 10	+3
5	May 31	-2	30	September 12	+3
9	June 3	-5	22	September 15	+3

This increases from 9 per cent. to 10 per cent. between the time of emergence and the first ten days of June, when it reaches a maximum. In undergoing this change the frog passes the midsummer weight about May 15. From this maximum the weight

TABLE IV

Containing twenty-eight records from leopard frogs (based on Table VII, Donaldson and Schoemaker, 1900). This table shows in successive columns the original tabular number, the sex, body-weight, length, and the observed and calculated weights of the central nervous system in milligrams. The last two columns show the percentage deviation of the calculated from the observed weights of the central nervous system; the percentages being computed on the observed values as the standard.

TABULAR NUMBER	SEX	BODY		WEIGHT OF CENTRAL NERVOUS SYSTEM IN MILLIGRAMS		PERCENTAGE DEVIATION OF CALCULATED FROM OBSERVED WEIGHTS	
		Weight in Grams	Length in Millimeters	Observed	Calculated	Deficiency	Excess
4	Female	5.06	102	68	63	7.3	...
5	Female	7.85	124	82 (c)	84	...	2.4
6	Female	10.90	136	103	99	3.9	...
7	Female	11.41	139	99	102	...	3.0
8	Male	12.30	141	106	105	0.9	...
9	Female	14.85	153	116 (c)	115	0.8	...
10	Male	16.28	164	122 (c)	121	0.8	...
11	Male	16.31	160	121	121	0.0	0.0
12	Male	17.13	166	122	124	...	1.6
13	Male	19.91	160	131 (c)	129	1.5	...
14	Female	20.35	162	130	131	...	0.7
15	Male	23.45	168	134	138	...	2.9
17	Male	27.42	172	142 (c)	146	...	2.8
18	Female	29.40	185	143	152	...	6.2
19	Female	30.45	179	141	152	...	7.8
20	Female	33.96	172	152	155	...	1.9
21	Male	36.03	198	171	164	4.0	...
22	Male	38.16	200	161 (c)	166	...	3.1
23	Female	42.54	215	177 (c)	175	1.1	...
24	Female	44.75	208	188	175	6.9	...
25	Male	45.37	205	174	176	...	1.1
26	Female	46.00	218	179 (c)	178	0.5	...
27	Female	47.58	206	191	178	6.8	...
28	Female	48.33	220	174 (c)	182	...	4.6
29	Female	52.55	206	180 (c)	182	...	1.1
30	Female	55.25	215	186 (c)	187	...	0.5
31	Female	61.10	228	193 (c)	194	...	0.5
33	Female	70.98	239	213	204	4.2	..
Average.....				146	146	2.9	2.5

Difference, 0 milligram; percentage, 0.0.

drops rather rapidly, about 5 per cent., to the end of June. In July and August it remains, with slight fluctuations, comparatively constant, and at the beginning of September falls off from 2 per cent. to 4 per cent. as the frogs enter upon hibernation. These observations of course apply strictly only to frogs subjected to the climatic conditions found in Chicago and the neighborhood within a radius of one hundred miles.

In the preceding Table III we have indicated the tabular number of the frog, the weight of whose nervous system has been corrected, the date at which the initial observation was made, and the amount of the correction. The correction is entered in this table as a percentage of the observed weight, the + sign indicating that the amount was added and the sign — that it was subtracted.

In Table IV the observed weight of the nervous system which is there given for these cases is the *corrected weight*, and the fact that it is a corrected weight is indicated by the small letter (*c*) which follows the entry.

The other records in Table IV are from frogs killed in July and August, and are therefore classed as midsummer frogs. It will be recalled that in the case of *Rana virescens* the formula for calculating the weight of the central nervous system is the same as that for the bullfrog, except that the constant, *C*, is 28 instead of 30. The formula reads, therefore:

$$C.N.S. = (\text{Log } W \times \sqrt[4]{L}) 28.$$

It is with this formula that the calculations appearing in Table IV have been made. The construction of Table IV is similar to that of Table I.

On applying to the records in Table IV the same tests as were used in the case of the bullfrog, Table I, we obtain results which are in some respects more satisfactory. It will be seen that the average weight of the central nervous system as calculated is exactly equal to the average weight observed. Further, if we divide the twenty-eight records into three groups of nine, nine, and ten, respectively, indicating the groups as A, B, and C, then the percentage differences for each group are seen to be also small — Table V.

TABLE V

To show the average percentage differences in the values of the observed and calculated weights of the central nervous system in three groups, formed from Table IV.

GROUP	TABULAR NUMBER	NUMBER OF RECORDS	PERCENTAGE DIFFERENCE; THE OBSERVED VALUES BEING TAKEN AS THE STANDARD	
			Deficiency	Excess
A	4-12	9	0.5	...
B	13-22	9	...	2.0
C	23-33	10	1.3	...

This shows a maximum deviation for Group B of 2 per cent., but it seems probable that a series of records based on averages would coincide more closely than this. On

examining in the leopard frog the percentage deviations, we find in one case (No. 11) exact coincidence, in twelve cases the calculated value is deficient, and in fifteen cases it is in excess. The average value of the deficiencies is 2.9 per cent., and of the excesses 2.5 per cent. These nearly balance, and point therefore to accidental causes as the source of the deviations.

These results for the leopard frog show that the curves fit somewhat better than in the case of the bullfrog, but the difference is not large nor significant. The statements that were made concerning the relations of the curves showing the observed and calculated weights in the bullfrog are also true for the corresponding curves based on the leopard frog.

It will be seen (Fig. 1) that in order to get the curves for the two species on the same chart, where they might be compared without being confused, the records for the leopard frog have been shifted 50 grams to the right. This enables us to see the approximate parallelism between the two curves, despite the fact that the leopard frog is differently shaped from the bullfrog, being somewhat more slender and having the relative weight of its trunk slightly less than that of the bullfrog (Donaldson, 1898, p. 334, Table IX; Donaldson and Schoemaker, 1900, p. 124, Table VIII). This slight difference in construction probably accounts for the necessity of using a smaller constant in the formula employed for the leopard frog, since the weight of the central nervous system would most probably be closely correlated with the development of the trunk. It is interesting to note, before leaving these records, that there is apparently no modification of the formula necessary for sex, in the case of either species. If we select the females from Table I, for the bullfrog, we find that they represent sixteen cases, or nearly one-half the number in the table. Arranging the records according to the tabular number, we have the percentage deviation for each case as given in Table I.

TABLE VI

Showing the percentage deviation for the female bullfrogs entered in Table I.

TABULAR NUMBER	PERCENTAGE DEVIATION		TABULAR NUMBER	PERCENTAGE DEVIATION	
	Deficiency, (7) Records	Excess, (9) Records		Deficiency, (7) Records	Excess, (9) Records
13	...	0.8	26	...	1.9
14	2.4	...	27	...	2.3
17	...	7.6	28	2.8	...
19	5.5	...	35	...	1.4
20	...	5.4	36	...	5.0
22	2.6	...	38	3.4	...
23	...	2.5	46	...	3.6
25	9.7	...	51	2.2	...

Average deficiency, 4.1 per cent.; average excess, 3.4 per cent.

On comparing the averages for the percentage deviations in these two columns we find the average for the excess 3.4 per cent., while that for the deficiencies is 4.1 per cent.—results practically the same as those obtained for both sexes in Table I.

TABLE VII

Showing the percentage deviation for the female leopard frogs entered in Table III.

TABULAR NUMBER	PERCENTAGE DEVIATION		TABULAR NUMBER	PERCENTAGE DEVIATION	
	Deficiency, (10) Records	Excess, (8) Records		Deficiency, (10) Records	Excess, (8) Records
4	...	7.3	23	...	1.1
5	2.4	...	24	...	6.9
6	...	3.9	26	...	0.5
7	3.0	...	27	...	6.8
9	...	0.8	28	4.6	...
14	0.7	...	29	1.1	...
18	6.2	...	30	0.5	...
19	7.8	...	31	0.5	...
20	1.9	...	33	...	4.2

Average deficiency, 2.9 per cent.; average excess, 3.9 per cent.

In this case the results are similar to those found in the female bullfrogs, except that the larger average percentage is on the excess side.

From these observations we conclude that in normal summer frogs of both sexes it is possible to calculate, with a high degree of accuracy, by the formulæ here employed, the absolute weights of the central nervous system.

Should others be inclined to test the correctness of these results by repeating the observations, it will be necessary carefully to avoid the sources of error which have been here enumerated, namely, the limitations of small size, the effect of season in the spring and autumn, and of the nutritive condition of the frog, as represented by starvation on the one hand, and the abnormal absorption of water or of drying on the other.

With these results in hand it is natural to seek for an interpretation of the formula which has been given in order to correlate it in detail with the changes which we know are going on in the central nervous system of the animals under examination. In the first place, the changes in the central nervous system, which the formula expresses, must take departure from the conditions which are present in the smallest frogs examined. In such a frog the central nervous system is composed of the supporting structures, neuroglia and ependyma, together with the blood and lymph vessels, and the nerve cells. These last form by far the greatest part of the substance. The nerve cells, or neurones, may be divided into those which are immature and those already

matured, in the sense that these latter have sent out both kinds of branches, dendrites and axone, and that the axone has acquired a medullary sheath.

As the frog grows larger the nervous system increases in weight. Concerning the method of this increase the following statements can be made: First, there is no cell division in either the supporting tissues or the nervous tissues at this time; hence the increase in weight is due to the enlargement of cell elements which are already present in the system. In the case of the neurones already developed and functionally active, this enlargement means an increase in the volume of the cell bodies, in the number and size of the dendrites, and in the length and diameter of the axone and its medullary sheath. In the case of the undeveloped neurones, it means a rather rapid acquisition of the branches and medullary sheath, to be followed by the slower changes just described above. In general, these changes tend to increase the complexity of the entire system, but, so far as they represent a mere lengthening of the connecting axones and a mere increase in their diameter, the added weight does not necessarily imply the increase in complexity, but only a passive adaptation of the system to the increasing size of the cavities in which it is contained.

The formula which we have employed indicates that where the body-weight is expressed by numbers increasing in geometrical progression, the weight of the central nervous system is expressed by numbers increasing in arithmetical progression, these latter being multiplied by a factor derived from the length of the entire animal.

Certainly this factor, depending on the length of the frog, is to be associated with the increase in the length of the brain and cord, but no satisfactory interrelation between this factor and this part of the growth process has been established. We are compelled, therefore, at this time to be content with pointing out the entire series of events which the formula expresses, without attempting to correlate any portion of the formula with any special part of the growth change.

#### SUMMARY

The formulæ here presented apply to the two species of frog: *R. catesbiana*, the bullfrog, and *R. virescens*, the leopard frog. The best results are obtained when frogs taken in midsummer (months of July and August) are alone used. The frog must be in normal condition and have a body-weight of 5 grams or more. When these conditions are fulfilled, then the weight in milligrams of the central nervous system (*C.N.S.*) of the bullfrog can be determined with a high degree of accuracy by the formula

$$C.N.S. = (\text{Log } W \times \sqrt[4]{L}) 30,$$

where  $W$  is the weight of the frog in grams,  $L$  the entire length in millimeters, and 30 a constant peculiar to the species.

In the same way the weight of the central nervous system (*C.N.S.*) in milligrams can be determined for the leopard frog by the formula

$$C.N.S. = (\text{Log } W \times \sqrt[3]{L}) 28.$$

This formula is similar to that for the bullfrog, except as regards the constant, which for the leopard frog is 28.

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#### EXPLANATION OF FIGURE I

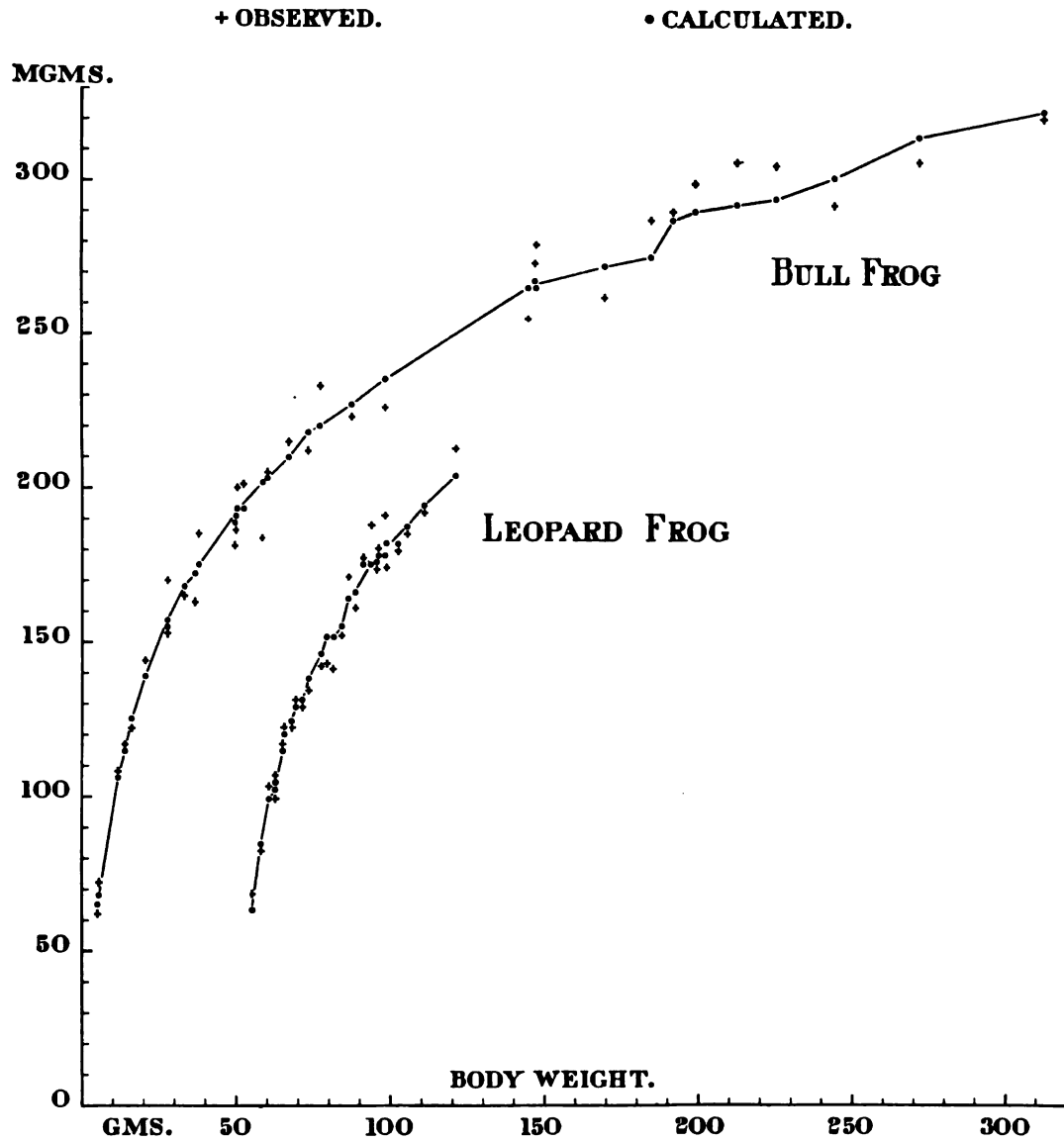
For this figure the data in Tables I and IV have been employed, and the accuracy of the figure can be tested by comparison with the tables.

The divisions on the base line indicate grams of body-weight; the divisions on the ordinates, milligrams of weight of the central nervous system. The observed weight of the central nervous system in milligrams is marked by + on the line of the ordinate above the point on the base line corresponding to the body-weight of the frog from which the central nervous system was taken. The calculated weight of the central nervous system is marked on the same ordinate line by a black dot (.). So far as it could be done without confusion, the (.) black dots indicating the calculated weights have been joined by a line, to better indicate their general relation to the observed records. The whole chart for the leopard frog has been shifted in the figure along the base line 50 grams to the right; hence all the body-weights in this chart are to be reduced by 50 grams from the weight indicated by their position.

In plotting the records, the indications (• and +) have been placed exactly where they belong except in those cases where exact placing would cause them to overlap. In such instances the displacement necessary for clearness has been distributed among the several records. In no case, however, does this displacement modify in any essential feature the character of the chart.



FIG. 1. WEIGHT OF BRAIN AND SPINAL CORD IN SUMMER FROGS



# **COLORS AND COLOR PATTERNS OF COLEOPTERA**



# THE DEVELOPMENT OF THE COLORS AND COLOR PATTERNS OF COLEOPTERA, WITH OBSERVATIONS UPON THE DEVELOPMENT OF COLOR IN OTHER ORDERS OF INSECTS

WILLIAM LAWRENCE TOWER

THE present contribution is limited, mainly, to an account of the colors and the development of the color patterns of Coleoptera, with some observations upon the colors and their development and chemistry in other orders of insects. As this research has been in progress for some time, the present paper does not aim to set forth in full either the data or the conclusions, but rather some of the more interesting results. On account of limited space, some observations upon the phylogeny of color patterns in Coleoptera, and an extended series of experiments made to determine the relation of various climatic and environmental factors to the production of variations, are wholly untouched. For the same reason the review of the literature and the discussion of data and results are reduced to a minimum.

I wish here to acknowledge my indebtedness to the Elizabeth Thompson Science Fund for a grant of money, which has been used in the experimental part of this research; likewise to Professor Franklin W. Hooper, of the Brooklyn Institute of Arts and Sciences, and to Professor C. B. Davenport, of the University of Chicago, for excellent privileges at the Biological Laboratory of the Brooklyn Institute of Arts and Sciences at Cold Spring Harbor, L. I., where much of the experimental work has been done.

In the course of this study observations have been made upon nearly all the groups of insects. Of these, species of the following list are either referred to directly in this paper or have contributed to the conclusions herein set forth:

PLECOPTERA	COLEOPTERA	<i>Tetraopes tetraophthalmus</i> Forst.
<i>Perla postica</i> Wlk.	<i>Carabus</i> . . . . <i>sp.</i> ? . . . .	<i>Leptinotarsa decemlineata</i> Say.
<i>Perla</i> . . . . <i>sp.</i> ?	<i>Hippodamia convergens</i> Guer.	<i>Pyrochoridus</i> . . . . <i>sp.</i> ? . . . .
NEUROPTERA	<i>Adalia bipunctata</i> Linn.	<i>Parandra brunnea</i> Fabr.
<i>Chrysopa</i> . . . . <i>sp.</i> ?	<i>Epilachna borealis</i> Fabr.	
<i>Myrmelion</i> <i>sp.</i> ?	<i>Cucujus clavipes</i> Fabr.	LEPIDOPTERA
TRICHOPTERA	<i>Anthrenus musæorum</i> Linn.	<i>Pieris rapæ</i> Linn.
<i>Gen. sp.</i> ?	<i>Psephenus lecontei</i> Lec.	<i>Clisiocampa americana</i> Fabr.
HOMOPTERA	<i>Alaus oculatus</i> Linn.	<i>Plusia brassicæ</i> Riley.
<i>Cicada tibicen</i> Linn.	<i>Chrysobothris femorata</i> Fabr.	HYMENOPTERA
HEMIPTERA	<i>Osmoderma scabra</i> Beauv.	<i>Comptonotus pennsylvanicus</i> De Geer.
<i>Anasa tristis</i> De Geer.	<i>Pelidonota punctata</i> Linn.	<i>Pelopoeus cementsarius</i> Dru.
ORTHOPTERA	<i>Orthosoma brunneum</i> Forst.	<i>Polistes pallipes</i> St. Farg.
<i>Phyllodromia germanica</i> Linn.	<i>Prionus</i> <i>sp.</i> . . . . ? . . . .	<i>Vespa maculata</i> Linn.
<i>Periplaneta americana</i> Linn.	<i>Phymatodes variabilis</i> Fabr.	<i>Bombus</i> . . . . ? . . . .
<i>Microcentrum</i> . . . . <i>sp.</i> ?		

Practically complete observations upon the development of the imaginal colors of these species have been made, and of many of them the larval coloration has also been studied.

The development of the color patterns was studied, and notes and drawings were made, almost entirely from living material, or, when this was not possible, from freshly killed specimens. Whenever possible, a series of stages illustrative of the development of the color patterns was preserved in several ways as an aid to the study of the development of the pigments.

For this purpose material was preserved in many of the usual fixing fluids—the corrosive-sublimate-acetic-acid mixtures, and Hermann's and Flemming's fluids giving excellent results. The corrosive-sublimate-acetic-acid mixtures were more uniform in action than the last two. Much valuable material was ruined by being preserved in the often recommended reagents for insects, such as Perenyi's fluid, hot water, alcohol, or corrosive sublimate. Hot water gave a good preservation of zymogen granules, but not as good as that given by 80 per cent. alcohol at 70–80° C. The picric-acid mixtures, except picric acetic acid and Perenyi's fluid, were too unreliable to be of use.

After being killed and fixed, material was usually preserved in 90 per cent. alcohol or imbedded in paraffin at once. All sectioning was done in paraffin. No reagents were used to soften the chitin, as, in my experience, no reliance can be placed upon material thus treated, and, although there was much breaking of sections, preparations that were sufficiently complete for the purposes of this research were not difficult to obtain.

All staining was done on the slide with Mayer's hæmalum, Delafield's hæmatoxylin, alum carmine, iron hæmatoxylin, thionin, and Kernschwarz's or Bensley's stain for zymogens.

#### HISTORICAL

The colors of animals are uniformly admitted to be due to one of the two following causes, or to a combination of these: first, to the existence within the body of the animal of a substance which has the property of absorbing all wave-lengths of light excepting a certain group which are reflected; or, second, to structural modifications of the surface which reflect, refract, or diffract the light. These two categories of color have been called respectively chemical and physical by Hagen (1883), and pigmental and structural by Poulton (1890); Mayer (1897) and others have adopted the latter terms, Mayer having added a third class, combination colors. As the categories of color established by Hagen, Poulton, and Mayer are co-extensive, on the grounds of priority Hagen's terms should have preference. Therefore Hagen's terms, "chemical and physical," will be used in this paper, and for Mayer's term, "combination," that of "chemico-physical" will be substituted for the sake of uniformity. These three categories of insect colors may be defined as follows: (a) Chemical colors are those which owe their existence to a substance, the product of the chemical activity of the body, which has the power of absorbing some light rays and reflecting others.

They are yellow, orange, red, buff, browns, black, and rarely green, blue, and white. (b) Physical colors are those which are produced solely by the action of surface structures, such as scales, lamellæ, pits, ridges, striæ, etc., which reflect, refract, or defract the light. They are the pearly colors, almost all whites, and rarely violets, greens, reds, and metallic and iridescent colors. (c) Chemico-physical colors are those which are produced by the combined action of chemical and physical causes. They are violet, greens, reds, and iridescent and almost all metallic colors.

It is probable that but few really pure physical colors will be found in insects, by far the larger part of those now classed as such falling into the category of the chemico-physical. Among the Coleoptera, in which I have made the most studies, I do not now know of a single case, excepting white, of an adult color which is a purely physical one.

Among insects the colors of Lepidoptera are the only ones that have been studied with any care, and what is known of the subject in this order is due almost entirely to the researches of van Bemmelen (1889), Hopkins (1889, '91, '94, '95), Mayer (1896, '97), Urech (1889, '90, '91, '92, '93, '97, '99), and Poulton (1884, '85, '86, '87, '90, '92, '93, '94). The colors of Lepidoptera have been divided by Poulton (1890) and Mayer (1897) into pigmental, structural, and combination—classes which are co-extensive with those of the preceding paragraph. Various criteria have been given by Dimmock (1883), Coste (1890–91), Urech (1893), and Mayer (1897) for determining whether a color is of a certain class. The solubility of the colors of Lepidoptera has been studied in a crude way by Coste (1890–91) and reported in a tedious and verbose paper. Further and more satisfactory contributions to the subject have been made by Hopkins, Urech, and Mayer in their numerous papers, and their results have been well summarized by Mayer (1896–97).

Little is known concerning the chemistry of the pigments or of their source. Landois (1864) found in the hæmolymph of several insects crystalline substances, egg albumen, globulin, fibrin, and several inorganic salts. Krukenberg (1884) found essentially the same substances, and recognized several pigments by their spectra. Hopkins (1889, '91, '94, '95) and Griffiths (1892) found white and green pigments, which they believed were uric-acid derivatives. From the researches of Zopf (1892–93) and others, reds and yellows are believed to be closely associated lipochromes, but their exact relations no one has been able to determine. Mayer (1896) has studied the hæmolymph of Lepidoptera, and finds present egg albumen, globulin, fibrin, xanthophyll, orthophosphoric acid, iron, potassium, and sodium. He believes that the colors are derived from the hæmolymph by chemical processes, and has performed some ingenious and interesting experiments, obtaining as a result substances whose color closely resembles that of the adult. Both Poulton and Mayer have shown the existence of derived plant pigments in the hæmolymph. The purity of the colors of the Lepidoptera has been investigated by Urech and Mayer. Pure colors are rare, or perhaps unknown, all colors thus far studied being composed of two or more compo-

nent colors; further, it appears from the researches of Mayer that relatively few simple colors are found in insects, the many and varied colors resulting from the combination of a few elementary ones.

In a valuable but much-neglected paper Hagen (1883) has shown that the colors of insects which are due to chemical activity are clearly divisible into two categories, known as dermal and hypodermal, which are sharply marked off from one another. Dermal colors are those which are found in the cuticula, and should more properly be called cuticula colors. The hypodermal colors are found in the hypodermal layer. I would add to these classes a third, the sub-hypodermal colors. Hagen has shown that the dermal or cuticula pigments are permanent, do not fade at death, and are very insoluble. The hypodermal colors are, on the other hand, not permanent, fade on exposure or at death, and are easily soluble. In the main these results are, I believe, correct. These classes, the colors belonging thereto, together with their solubility and permanence according to Hagen, are briefly described by the following table:

CHEMICAL COLORS. TABLE OF COLORS, THEIR SOLUBILITY AND PERMANENCY. (HAGEN)

Dermal colors = <i>Cuticula colors</i>	{	Yellow	-	-	-	} Are permanent, do not fade at death, are insoluble, without dissolution of the cuticula, in water, acids, alkalies, alcohol, ether, or essential oils. Soluble in strong mineral acids with dissolution of the cuticula.
		Brown	-	-	-	
		Black	-	-	-	
		Drab	-	-	-	
		Red?	-	-	-	
Hypodermal colors	{	Red	-	-	-	} Not permanent, fade at death and on exposure. Easily soluble in usual organic solvents.
		Yellow	-	-	-	
		Orange	-	-	-	
		Green	-	-	-	
		Blue	-	-	-	
		White?	-	-	-	

No other author has, in my opinion, so clearly set forth the character of insect colors as Hagen, and outside of the Lepidoptera his work still remains the only comprehensive survey of coloration in insects.

The ontogenetic development of color patterns in Lepidoptera has been studied in much detail by van Bemmelen (1889), Urech (1891), Hasse (1893), Mayer (1896, '97), von Linden (1898, '99, 1901, '02), and others, and they uniformly agree that the wings—they have not studied the development upon the body—are at first colorless or nearly so; that they soon become white or opaque; then of a yellowish or drab color, which, beginning at the base of the wing, spreads over its entire surface. After this the adult color, spots, and stripes begin to appear and go through a rather regular series of changes before arriving at the adult condition. The general relations of the colors and the development of markings can be briefly and accurately shown by the following table:

WING STAGES	COLORS
I. Colorless	Colorless or transparent
II. Opaque	<div style="text-align: center;">           Hæmolymph enters            white   cream-white   yellow.   green         </div>
III. Monochromatic	<div style="text-align: center;">           drab   yellow   white   yellow   green         </div>
IV. Ground colors	<div style="text-align: center;">           drab   yellow   white   orange   brown         </div>
V. Markings	<div style="text-align: center;">           drab   grey   (light) red   brown            grey   (dark) red   crimson   black         </div>
VI. Physical color	<div style="text-align: center;"> <u>silvery</u>   <u>golden</u>   <u>pearly</u>   <u>metallic colors</u>   green         </div>
VII. Adult	Adult colors are underlined so as to show when each reaches its mature condition

Derived plant pigment in solution in the Hæmolymph

In the other orders of insects only the most fragmentary observations have been made upon color development; hence any attempt at a summary is impossible.

### Colors of Insects

It appears from what has been said in the preceding pages that the colors of insects are clearly divisible into certain categories, each having well-defined characters. It further appears that the colors of Lepidoptera are the only ones that have been at all well studied, and even in this order only those of the wings have received attention. In this section it is proposed to examine into the location, character, and arrangement of the different colors forming patterns in the various groups of insects. This has already been partially done by Hagen (1883), and to his work I shall constantly refer.

In his survey of this subject Hagen recognized, lying in the cuticula, a group of chemical colors which he called dermal. They had the permanency and general characters already pointed out above. Later, Poulton recognized "colors proper to the species" which were modified only slightly, or not at all, in his experiments. These colors lie entirely within the cuticula and are black (?), dark brown, brown, rufous brown, yellow, and possibly some reds. They are situated entirely in the outer or primary layer of the cuticula, and in section present a uniformly colored appearance, even in the thinnest of sections. This location and character of the color is constant for many, if not all, orders of insects, as shown by the figures of the body wall of *Anasa tristis* (Pl. III, Fig. 79), *Chrysobothris femorata* (Pl. III, Fig. 61),



*Leptinotarsa decemlineata* (Pl. III, Figs. 64 and 65), *Orthosoma brunneum* (Pl. III, Fig. 69), *Clisiocampa americana* (Pl. III, Figs. 70 and 71), and *Polistes pallipes* (Pl. III, Figs. 72 and 73). This coloring substance Hagen believed to be deposited in the form of small granules in the cuticula, but sections  $3\frac{1}{2}\mu$  in thickness and the best optical appliances fail to show this to be true. They are, on the contrary, diffuse colors.

From the figures given it appears that this colored layer, which is so permanent in character, is in the outermost layer of the body wall, and is separated from the living, actively growing tissue by the secondary cuticula (Pl. III, Fig. 70 cta.<sup>2</sup>), which is colored only very rarely, and then only in the latter part of the adult life. Most of the cuticula colors are browns or blacks, yellows being relatively uncommon. On looking over the orders of insects, excepting the Lepidoptera, these cuticula colors are found to predominate to a large extent, at least 95 per cent. of all patterns being dependent upon them for their most characteristic markings, and at least 75 per cent. for their entire coloration. They are, therefore, of paramount importance, and to their origin, both ontogenetic and phylogenetic, I shall devote a good share of the latter part of this paper.

The relation of the cuticula colors to the hypodermal or sub-hypodermal in the production of markings or patterns is fundamentally that the hypodermal color acts as a groundwork upon which the pattern is cut out by the cuticula color, in the same way that a fresco painter lays darker colors over the lighter ground-color to produce the mural decoration. The relation of the two colors is shown in section in the larva of *Clisiocampa americana* (Pl. III, Fig. 71), the section being taken through one of the reddish spots upon the dorsal surface. The cuticula color, which is uniformly distributed over the surface of the body, is broken by a transparent place through which light may pass to the red hypodermal color below and thus produce the spot. When the cuticula color is widely diffused over the surface, this arrangement gives red or yellow spots or stripes in a general body color of brown or black; or, if the cuticula color be limited to small areas, the insect is yellow or red with brown or black spots. If one will take the trouble to look over a large collection of insects, he will find that these are the dominant colorations in all orders.

The hypodermal colors, as recognized by Hagen, were not permanent and were easily soluble in ordinary weak reagents. This, while partially correct, is not entirely so. There are two well-marked classes of hypodermal colors: one group, which exists as definite granules in the cytoplasm of the hypodermal cells, includes those colors that are permanent, in that they do not fade at death and are not soluble in water, alcohol, ether, essential oils, weak acids, or alkalies. They are vermilion, scarlet, most, if not all, chemical reds, and chrome yellows. These colors, which were mistaken by Hagen for dermal colors, are shown in section in *Clisiocampa americana* (Pl. III, Figs. 70 and 71), *Anasa tristis* (Pl. III, Figs. 77, 78, and 79), and *Coccinella* (*Adalia*) *bipunctata* (Pl. III, Fig. 76). The hypodermal colors of the second group are yellow

and green, diffuse in their nature, and are plainly the derived plant colors, xanthophyll and chlorophyll, taken from the food. They are not permanent, sections being obtained only by the freezing methods. They exist in the hypodermal cells as well as in the hæmolymph below in the body cavity, their location being shown in the section of *Epilachna borealis* (Pl. III, Fig. 75). These colors are entirely characteristic of phytophagous species, excepting the carnivorous Coccinellidæ, whose food in many cases is the hæmolymph of aphids, which is heavily laden with these derived plant pigments. Further, these colors are characteristic of the larva. There is not, to my knowledge, any case in the Holometabola, outside of the Lepidoptera and Chrysomelidæ, in which a derived green or yellow forms part of the imaginal coloration, the fairly numerous adult yellows being lipochromes located in the hypodermal cells.

The greater part of the hymenopterous, dipterous, and coleopterous larvæ show no trace of dissolved plant pigments or of other hypodermal colors. They are white, cream, rarely yellow or red, their coloration being due to the fat body or to the food in the intestine. The sub-hypodermal colors are purely larval in character and are not of any great importance.

Physical colors are not, as I have before stated, of as common occurrence in insects as might at first thought be supposed. I do not know of a single case of a *purely physical color* excepting white. They are produced by surface modifications which reflect, refract, or defract the light in the same way as would physical appliances. The production of color by such means is fully discussed in physical literature and need not occupy space here.

The chemico-physical, or combination, colors of Mayer (1897), which are of exceedingly wide occurrence, are also the most brilliant and varied of all those found in insects. To this class belong all metallic, iridescent, pearly, and translucent colors, as well as blue, green, and violet in almost every case.

The simplest of these chemico-physical colors are undoubtedly metallic blue, green, and golden (yellow), which are produced by a lamella with a pigmented layer below. The lamellæ are thin, highly refractive, and have the outer surface polished. As a result, light impinging upon this surface is in part reflected as white light, while the remainder penetrates and is bent in such a manner that certain rays are again reflected from the lower surface of the lamella, and these are the rays which give color to the structure, *i. e.*, blue, green, etc. A large part of the light passes through the lamella into the pigmented layer below, where, as it passes through, part is absorbed and the rest is reflected from the deeper layers. It undergoes a second absorption in passing out from the body, so that by the time the light has traversed the pigmented layer a second time relatively little is left to emerge through the lamella, if it emerges at all; for in many cases the lower surface of the lamella acts as a mirror, reflecting back into the pigmented layer the light returned from below. In all of the Coleoptera that I have examined, and in every other case of metallic blue or green that has

come under my observation, there is found, below the reflecting lamella, a dense, dark, absorptive layer, whose function is obvious. If a portion of this absorptive layer be removed, less of the transmitted light is absorbed, and more comes back to the surface and there blends with the reflected and refracted colors, thereby weakening them.

The quality of the blue or green depends upon the degree to which the surface is polished, the refractive index of the lamella, and the completeness of the absorption of transmitted light. If the refractive index is low, that is, if only a small amount of light is refracted, and if absorption is almost complete, the resulting color will be a deep blue-black, like that on the ventral surface of *Pelidonota punctata*, or a greenish black, which is common on many species of Coleoptera and Hymenoptera.

Golden or metallic yellows are produced in much the same way. There is a polished surface on the outside which reflects a small amount of white light to give brilliancy; a lamella, whose refractive index is such that only certain yellow rays or none at all are refracted; and a layer of yellow cuticula or yellow hypodermal color below. This yellow light, passing out from below, mingles with the surface reflection and gives a metallic or burnished appearance. Greenish gold, reddish gold, etc., are produced as above, excepting that the outer lamella refracts a greater or less amount of red or green rays, thus adding the extra tint to the coloration.

Sections of the body wall of species showing metallic colors never have any pigments excepting cuticula browns and yellows and hypodermal yellows and reds, the two latter being of rare occurrence. The arrangement of the lamella, absorptive layer, and lower reflecting layer is shown in the section of *Chrysobothris femorata* (Pl. III, Fig. 60).

The most complicated of the chemico-physical colors are the iridescent, which occur in an endless variety of tints and combinations. In the Lepidoptera, Kellogg (1894), Mayer (1897), and others have shown that striae are a potent cause of iridescent colors, but in Coleoptera, as also in other orders, striae are rare even upon scales. A common cause of iridescence is the existence of fine, closely set pits, as pointed out by Hagen (1883). The pits alone, however, are powerless to produce any color; it is only when they are combined with a highly reflecting and refractive surface lamella and a pigmented layer below that the iridescent color appears. The action of light is in this case the same as in the plain metallic coloring, excepting that each pit acts as a revolving prism to disperse different wave-lengths of light in different directions, and the combined result is iridescence. The existence of minute pits over the body surface is of common occurrence, but it is only when they are combined as above that iridescent colors occur.

To the category of chemico-physical colors belong many yellows, as, for example, the yellow of the body of certain species of *Bombus*. In these species (*Bombus*) the cuticula color is well developed as a deep brown; over this is a thick layer of highly refractive scales which reflect a large amount of white light, and the combined result of the cuticula color and reflected white is yellow. In Coleoptera, many of the scaled

species have such yellows or yellow-browns which owe their existence to exactly similar causes. That the powdered, downy, or bloom appearance of many beetles is due to scales has already been shown by Dimmock (1883). In Lepidoptera I suspect that many of the yellows and browns are produced by this same cause.

From the preceding statement and examples, it appears that of all colors in insects those of the cuticula are of the greatest importance; for not only do more than half of the colors of insects belong directly to this class, but nearly all of the remainder are made possible by this layer of colored substance which acts as the backing to a mirror to reflect or absorb, as the case may be. Without this action we should have few if any metallic or iridescent colors. The hypodermal colors are, moreover, not of wide distribution, but are confined to certain families. They are essentially larval colors and as such play large rôles in larval color patterns. Of all colors white is probably the only purely physical one, and it is probably to this class that the greater part of the adult whites belongs. In larvæ, however, white is almost entirely a sub-hypodermal color due to the fat body, and larval and adult whites are thus quite different colors, so far as cause is concerned. The relation of the various colors may be conveniently shown by the following table:

Chemical colors	(a) Cuticula colors	{ Black Dark brown Brown Straw yellows }	Located in primary cuticula	{ Permanent. Insoluble in water, alcohol, ether, oils, weak acids, or alkalies Soluble in strong concentrated mineral acids with dissolution of the cuticula }
	(b) Hypodermal colors	{ (1) { Chrome yellows Red Vermilion Scarlet Blue } (2) { Green Yellow White } }	Located in hypodermal cells as granules	{ Lipochromes } Permanent. Insoluble in water, oils, alcohol, weak acids, or alkalies Soluble in ether or other fatty solvents
	(c) Sub-hypodermal colors	{ Green Yellow White }	Located in or between the hypodermal cells	{ Derived pigments } Not permanent. Fade at death or on exposure Soluble in water, alcohol, etc. Are chlorophyll or xanthophyll derivatives largely
Physical colors	(d) Reflection colors	{ White }	Caused by air included within scales, etc. Most common, and perhaps the only true physical color	
	(e) Refraction colors	{ Metallic colors }	See next class	{ Opalescent colors } Caused by combining white and some metallic refraction color, usually with pigment present. Frequently caused by their irregular lamellæ over pigment, giving effect of Newton's rings
	(f) Defraction colors	{ Iridescent colors }	See next class	

Chemico-physical colors	(g) Reflection pigmental colors	{ Colored surfaces with polished appearance	{ Blacks Browns Yellows Reds	{ Caused by a polished lamella surface over layer of pigment
	(h) Refraction pigmental colors	{ Almost all metallic colors	{ Cause — Polished refractive	{ lamella overlying a layer of pigment
	(i) Defraction pigmental colors	{ Almost all iridescent colors	{ Cause — Surface structures, pits, ridges on refractive lamella overlying a layer of pigments	
	(j) Combination colors	{ Various iridescent metallic, opalescent metallic colors, etc., in which colors of groups g, h, and i combine to produce color effects	{ This class of color is confined largely to Lepidoptera and almost exclusively to scaled insects or areas bearing scales	

### *Ontogeny of Color Patterns*

On a previous page it has been pointed out that the ontogenetic studies of the color patterns of Lepidoptera have dealt exclusively with the wings. I have been chiefly interested in following the color development of the body, to which no attention has been given, and for this purpose the species of Coleoptera mentioned above have furnished excellent material. The following account, therefore, deals mainly with color-pattern development in Coleoptera, with some discussion of the same in other orders of insects. The general features of color development are so nearly alike that the Coleoptera serve admirably for the purpose of illustration. Moreover, the very ancient character of the group makes it possible to demonstrate several rather generalized conditions of color development.

*Coleoptera*.—Abundant material of the large Cerambycid beetle, *Orthosoma brunneum*, was obtained from decaying logs at Cold Spring Harbor, L. I., every stage in its ontogeny being found. On Pl. I, Figs. 1-11 inclusive, I have shown the general features of the color development of this species. The short pupal stage of about three weeks is followed by an adolescent stage, which is passed in the pupal cell, when the colors mature and the body wall and appendages gain the necessary firmness to enable the beetle to exist in the outside world.

The pupa is shown in Fig. 1 (Pl. I) at about two weeks after pupation. No color has appeared upon the body excepting the pigment of the eyes, which can hardly be called part of the color pattern. Soon color appears upon the head — first upon the mandibles, then upon the other mouth-parts and the epicranium. This color, which is at first yellow, soon changes to yellowish brown, brown, dark brown, and may even become so dark as to appear black. The first appearance of color upon the head is over the attachment of the muscles to the cuticula. From these centers it spreads until the whole surface is of a uniform yellow brown (Pl. I, Fig. 2). In development, color first appears over a  $\perp$ -shaped-area in the median line, which extends also along the posterior border of the epicranium (Pl. I, Fig. 2). Later it appears lateralward from this, but soon becomes fused in the general brownish yellow which covers the

part. After attaining a uniform brownish yellow, the whole head gradually becomes brown, then a deeper brown which may in some specimens appear almost black (Pl. I, Fig. 3).

On the pronotum color first appears as a  $\perp$ -shaped area, with the long arm of the  $\perp$  in the median line and the short arm extended lateralward along the posterior border of the pronotum (Pl. II, Fig. 23). Following this stage, color appears lateralward of the median line, first close to the median line as an elliptical area with the long axis in an antero-posterior direction, and then as two areas near the outer border of the part (Pl. II, Fig. 24). These centers develop color rapidly, and two additional areas appear anteriorly to the first pair of spots; from these centers color spreads rapidly in all directions (Pl. II, Fig. 25) until a general yellow brown tone suffuses the entire surface, with the centers of coloration, which are over muscle attachments, appearing as darker nuclei (Pl. II, Fig. 26). The general ground color now darkens and eventually attains a shade as deep as that of the centers from which the coloration spread, so that these are now obliterated and the entire surface presents a uniform color (Pl. I, Fig. 4, Pl. II, Figs. 27 and 28).

Upon the ventral surface color has lagged behind, being by this time of a light yellow-brown, with a slightly darker tinge on the coxæ, on the proximal ends of the femur and tibia, and over muscle attachments to the ventral body wall. On the ventral abdominal surface areas of faint yellowish color have also appeared over the muscle attachments (Pl. II, Fig. 43), and the legs and antennæ have developed a uniform dark yellow, with traces of deeper color over the muscle attachments (Pl. I, Fig. 4). While about in this stage the beetle emerges from the pupa, the wings expand, and color development goes on again in the adolescent stage. The condition of the beetle just after the completion of its transformations is shown in Fig. 4 (Pl. I). At this time the head and dorsal surface of the prothorax have developed the mature color; the ventral thoracic surface may rarely have reached adult coloration, but it is usually only about half developed; the wings and dorsal abdominal surfaces are creamy white; the ventral abdominal surface has traces of color as shown in Fig. 43 (Pl. II), while on the legs and antennæ it is only partially developed (Pl. I, Fig. 4).

In the adolescent beetle (Pl. I, Figs. 4-11) the color development consists mainly in the changes in the elytra and the completion of the pattern over the rest of the body. The elytra, which are at first white or creamy (Fig. 4), become yellowish (Fig. 5) and then yellow brown (Fig. 6). Eventually a darker brown begins to appear (Fig. 7), beginning at the anterior end and growing posteriorly until the entire wing is nearly covered (Fig. 8). This is followed by darker shades spreading over the wing in the same manner (Figs. 9 and 10) until the adult color is attained (Fig. 11). The legs, antennæ, and abdominal surfaces also pass through a similar series of stages (Pl. I, Figs. 5-11; Pl. II, Figs. 43-5).

Thus the sequence of color in this beetle is: first, the sub-hypodermal color, creamy white, which is derived from the larva and pupa; then the pale yellow cuticula

color, followed by yellow, brown, and finally by dark brown, which is often so dense that it appears black by reflected light. In the color pattern only cuticula colors are found. In the development of color, centers of coloration first appear over the muscle attachments and from these spread in all directions. Further coloration begins at the anterior end of the body and progresses posteriorly, and the same is true of every part. The ventral surface in general lags slightly behind the dorsal surface in development, although the dorsal abdominal surface is retarded most of all.

In *Parandra brunnea* (Spondylidæ) the adult pattern is entirely of cuticula color, and in its ontogeny passes through stages almost exactly like those described and figured for *Orthosoma brunneum*, excepting that a light yellow-brown appears upon the elytra before the insect leaves the pupa. The color of the elytra is developed first in intertracheal spaces, as stripes running longitudinally, but before emergence all stripes coalesce to form a uniformly tinted surface. These stripes develop and fuse from before backward.

In *Osmoderma scabra* (Scarabæidæ) color appears on the body in exactly the manner described and figured above for *O. brunneum*, but requires a much longer time in which to complete its development. The elytra in the pupa show faint drab stripes between the tracheæ, which, however, become confluent at about the time of emergence.

In *Carabus*—sp.?—and *Pterosticus*—sp.?—the color pattern develops over the body in the same manner as described above, excepting that on the pronotum the spotted stage is extremely short, and in some series seems altogether lacking. Moreover, the wings become drab before the beetle emerges from the pupa, and are without any trace of stripes that I can observe.

From the preceding paragraphs it appears that beetles with a color pattern of one cuticula color develop in almost exactly the same manner, and that there is practically no variation excepting upon the wings. As the wings are less essential parts of the body wall and phylogenetically much younger, more variation of color development on them might be expected.

In *Leptinotarsa decemlineata* (Pl. I, Figs. 12-21 incl.) is shown another type of color-pattern development composed of a dark cuticula pigment upon a yellow hypodermal background. Like the preceding species, this beetle develops only part of its color in the pupa, the remainder being formed in the early part of the imaginal stage. Both, however, have many features in common.

The general trend of color-pattern development of this species is shown in Figs. 12-21 (Pl. I), where the pupa is represented as being straightened out. The spots shown upon the abdominal surface (Pl. I, Figs. 12-14) have no relation to adult structures or markings. They are pupal markings, indicating the location of immense thickenings in the cuticula which lie over segmentally arranged hypodermal thickenings or discs. At first the pupa is of a rather bright yellow, due to the existence of a large amount of xanthophyll in the hæmolymp, but it soon becomes lighter in

color (Pl. I, Fig. 12). About five days before the final transformation the eyes have attained their full quota of pigment, and the color pattern has begun to appear upon the head, first upon the mandibles and epicranium and then upon other parts, as faintly colored yellow-brown areas lying over muscle attachments. Upon the epicranium two oval spots appear lateralward from the median line, and a crescentic spot in each posterior outer angle. Soon, however, the two central spots fuse across the median line, forming a heart-shaped area (Pl. I, Fig. 13). Often there is a caudalward and lateralward spreading of the pigmented central area, which then becomes united with the two crescentic areas and forms a large, deep brown spot which covers nearly the entire upper surface of the epicranium.

About three days before emergence color appears, first upon the pronotum (Pl. II, Fig. 36, ii) in the median line and then in a large oval area lateralward (Pl. II, Fig. 37, iii, iv), and in the outer angles (iv, v). The further change which occurs in the pupa consists in the darkening of the pigmented areas until they reach the

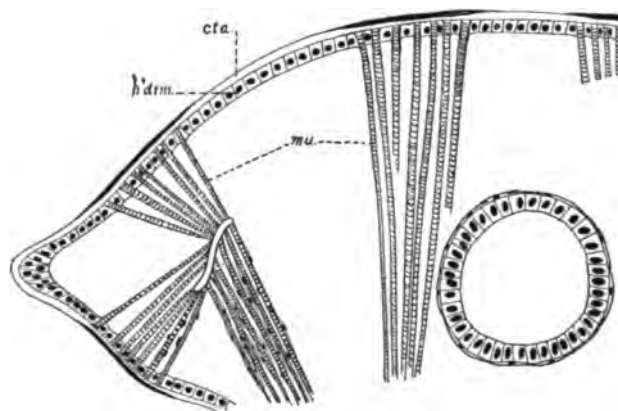


FIG. 1

adult deep brown just before emergence (Pl. II, Fig. 39). The series, Figs. 35-42 incl. (Pl. II), is intended to illustrate the development of the color pattern of the pronotum. Each of the spots upon this part is developed in connection with a muscle, and marks the point of attachment of its fibers to the cuticle, as shown in the diagram (Text Fig. 1). Figs. 41 and 42 present interesting stages in the development of the color pattern of an extremely melanic specimen, as they show the caudalward and lateralward spreading of pigment over the surface in much the same lines as that observed in *Orthosoma brunneum* and figured on Pl. II, Figs. 23-8 incl.

In the development of color upon the ventral surface of the body there is found in *L. decemlineata* the retardation observed in *Orthosoma brunneum* and other species, excepting that in this species the development of the ventral color pattern is more variable, and it may have reached a considerable degree of advancement at the final transformation, or it may have just begun to appear. Whether it develops early or late, the features of development are in no manner different from those described for the previous species. The pattern, as it usually exists at emergence, is shown in Fig. 15, Pl. I.

In the newly transformed imago the elytra show no markings of any kind excepting the uniform pale yellow color and the rows of impressed punctations (Pl. I, Fig. 15).



About a half-hour after the elytra are fully expanded they assume a brighter yellow color, and the spaces between the rows of punctations show a yellow-brown tint, which marks the beginning and location of the future longitudinal stripes (Pl. I, Fig. 16). This yellow-brown color usually begins to appear first at the anterior end of the elytron and grows posteriorly very rapidly, though in many specimens it seems to appear simultaneously along the entire length of the stripe. About one hour after emergence the elytra are decidedly yellowish, and the stripes have become distinct and light brown in color. The increased yellow is due to two causes: first, to the accumulation of a yellow "derived" pigment which is brought into the elytron with the hæmolymp; and, second, to the development of a yellow color in the cuticula (Pl. I, Fig. 17). Later stages of the color development are shown in Figs. 18 and 19 (Pl. I), the latter being the imago as it appears when it first emerges from the ground. In Fig. 20 (Pl. I) is shown a condition produced in some temperature experiments where a reddish tint has developed in the hypodermal color due to a lipochrome, which, however, is not permanent. Another line of modification is shown in Fig. 21 (Pl. I), where the hypodermal color is almost entirely absent.

*Coccinella bipunctata* (Coccinellidæ) is interesting as showing the development of color in a species almost of one uniform yellow tint. In the pupa color develops over the head, thorax, and ventral surface in much the same way as in the preceding form, but the wings show no change until after emergence and expansion. When first transformed the wings are of a pale cream color, but as soon as the hæmolymp enters they become yellow as a result of the dissolved xanthophyll which the hæmolymp carries. Soon, however, a yellow color suffuses the wing, which is different from the hypodermal yellow and is due to the development of a yellow color in the cuticula. At about the same time the black spots begin to appear and follow much the same order of color change as has been described before. The point of interest here is the groundwork of yellow, which is due to two causes: the faint yellow of the cuticula and the strong yellow of the hypodermal colors. This ground color is extremely variable. It ranges from pale yellow to a deep red, depending upon the conditions of the hypodermal pigments. It has been suggested that the development of red from yellow and yellow from red is due either to the addition of a radicle to the color-producing molecule, or to there being two closely associated lipochromes which always occur together, and that the yellow or red condition marks the ascendancy of one over the other (Zopf, 1891).

In *Pelidnota punctata* the same general features of color development are found, excepting that here there are no hypodermal colors. The wings (Pl. II, Figs. 51-3) are at first pure white, but become yellowish by the development of the cuticula yellow. This color is further reinforced by the polished surface of the elytron which, by reflecting many white and yellow rays, produces a strong yellow or yellow-brown. Color develops upon the ventral surface rather precociously in this species, so that the adult color is attained before the imago emerges.

The species described above all develop a considerable portion of the adult coloration after the insect has left the pupal coverings, and this is true for beetles as a group, though not for all beetles. Thus, *Epilachna borealis*, as well as several other species of Coccinellidæ, emerge from the pupa with the colors fully formed. Almost all of the beetles of which scales form part of the color pattern develop much, if not all, of their color before leaving the pupa. The color of the scales of beetles is, so far as I know, always of cuticula or of physical origin, although it is probable that lipochrome pigments will be found in the scales of Coleoptera as in Lepidoptera.

It appears that many of the white and yellow markings of the wings of beetles are due to scales (Dimmock, 1883). White is usually a structural effect, but it may sometimes be pigmental, as in *Arthia punctata*, *Graphypterus serrata*, and *Scæitus polyphemus*, from Algiers, in all of which the clear white color of the spots is due to a white substance within the scales. As to the nature or development of this white coloring matter I have not the necessary material to make a study. In many Cerembycidæ, as *Cyllene robinæ* or *C. pictus*, the yellow markings are composed of scales carrying a yellow pigment, which is probably entirely cuticula; at least it is not changed at death or by weak reagents, as hypodermal pigments are apt to be. The eye spots of *Aulus oculatus* are formed by a patch of deep brown (black) scales, surrounded by a ring of white ones. In this case the black is a cuticula color and the white largely, if not entirely, physical, as determined by Dimmock's tests.

In the beetles which have patterns formed by colored scales (Pl. II, Figs. 46-50) the wing below is always, so far as I have observed, of a uniform color, usually dark. If one removes the colored scales with a sharp knife, the deep brown cuticula color is exposed. In the Coleoptera the colors of the scales—yellow, brown, and black—are exceedingly stable and are cuticula colors, as are the same colors in species without scales. In Lepidoptera Coste (1890-91), Urech (1893), and Mayer (1896) show that in general yellows, browns, and blacks, especially the two latter, are very stable, and it is probable also that they are cuticula colors.

Many beetles have metallic or iridescent colors superimposed upon the cuticula or hypodermal color pattern. On a previous page I have described how these metallic, or chemico-physical, colors are produced, and the mechanism thereof; it now remains to describe the development of the pattern to which these metallic colors belong. Excellent material for this is found in the Buprestidæ. In *Chrysobothris femorata*, stages in the ontogeny of the color pattern are found which are like those of other Coleoptera. In the pupa color first appears upon the mouth-parts and over the attachments of the head muscles, and from these nuclei spread over the entire surface. The color sequence is the same as that observed for other species where the cuticula colors develop, hypodermal color being absent in the Buprestidæ. Upon the pronotum, for example, the development of the color pattern is similar to that of other species, and a comparison of the figures of this species (Pl. II, Figs. 29-34 incl.) with those of others will show the points of similarity.

In the development of the color, however, as soon as the cuticula pigment has reached a sufficiently dense stage to be of use as an absorptive layer, the metallic colors begin to appear. This I have shown in the four figures of the elytra as representing a simple condition (Pl. III, Figs. 54-7 incl.). The elytra, which are at first nearly white, become darker, as shown in the figure, until the pigment is able to absorb sufficient light to make visible the refraction of the surface lamella, which then appears as a greenish sheen. In later development the surface becomes ridged, and the absorption more complete, so that stronger colors are produced. In the adult the color is dark brown, with metallic blue, green, and gold colors displayed in varying degrees as the relation of the light to the surface changes.

From the foregoing account of the ontogenetic development of color patterns in Coleoptera it appears that all have certain important features in common, and upon these minor characters are superimposed. We recognize that the cuticula colors are the most important in this order, and that in ontogeny they appear at the anterior end of the body, first over the attachments of muscles or upon important sclerites, and then in a rather regular way spread posteriorly, although precocious development of coloration in a part is frequently found. From these centers as nuclei colors spreads over the surface until the adult condition is attained. For this reason the possibility of diversity in color pattern upon the body of beetles is limited to more or less segmentally arranged series of spots or stripes, either condition being derived from the primitive color nuclei by fusion or otherwise. It should be noted that color is not restricted to the muscle attachments, but may exist as spots in areas free from muscles. In these cases, however, a study of the phylogeny of the color pattern shows that it has probably been derived from an ancestor which had the entire part colored, and the spot persists as the color is resolved into its component nuclei. These spots are, moreover, the most highly variable of all the color markings. It is also to be noted that the muscle attachments are the last areas to lose their color and the first to regain it.

Upon the wings color patterns are not so closely correlated with deeper structures, and thus a greater diversity is possible; yet even here the pattern tends to be arranged in stripes or rows of spots, being either longitudinal between the veins, or transverse bands at definite places in the wing.

Some beetles have no cuticula colors, but these are rare and unimportant. The hypodermal colors in Coleoptera are essentially larval, and are confined largely to phytophagous species which derive their hypodermal color from the derived plant colors xyanthrophyll and chlorophyll. Exceptions to this are found in the carnivorous Coccinellidæ, which, as they feed upon phytophagous species, derive the colors secondarily. The metallic and physical colors are younger ontogenetically, as probably also phylogenetically, than either the cuticula or hypodermal color.

At the beginning of this section I stated that the general features of color-pattern development found in Coleoptera are likewise the general features of color-pattern

development in other orders of insects, and I shall now attempt to show that this is true by describing the color development in some other orders of insects, not, however, going into such detail or giving as many examples as have been given for the Coleoptera.

*Plecoptera*.—Owing to the absence of a pupal stage in this order, observations upon its color-pattern development must be confined to the changes either of the larva at ecdysis or of the imago at emergence. In either case immediately after the insect leaves its old cuticula covering it is pure white and very soft and delicate, with black eyes and ocelli. About a half-hour after ecdysis is completed color begins to appear upon the head as paired spots lateralward from the median line, then upon the mandibles, and later upon the other mouth-parts and head. These spots, which are at first a pale yellow-brown, grow darker, reaching their adult condition in about six or eight hours.

Upon the thorax color appears almost simultaneously as streaks or bands over the muscle attachments of the legs and body, and as diffuse color upon the wing-pads of the nymph. These develop rapidly to the adult condition. The color of the abdomen spreads out over the part from a series of segmentally arranged spots. The entire coloration is completed in about ten hours after ecdysis.

In the imago the development does not differ greatly from that of the nymph, excepting in the wings. On the body color appears over muscle attachments and spreads from these as centers. Parts uniformly colored in the nymph may have spots or bands in the adult, brought about by the breaking up of a uniformly colored area. These spots or stripes always bear a definite relation to the muscles, so that, in this order as in the Coleoptera, the location of muscle attachments to the cuticula is a potent factor in the determination of body color patterns, and, although dark spots and markings are found which are not associated with muscle attachments, still their most characteristic and noticeable features are so associated.

*Neuroptera*.—The dark markings of the adult in this order do not differ in their development from those of the preceding one. They are associated with the muscle attachments, but have less relation to the larval coloration than those in the Plecoptera. In Chrysopa the green of the body is due, in part, to secondarily derived colors. The larva feeds largely upon phytophagous insects, sucking from them the hæmolymph with its dissolved pigments. These persist in the larva, pupa, and imago of Chrysopa and give a greenish color to the body, which, taken in connection with the effects produced by the surface lamellæ, produces brilliant green and golden colors.

*Trichoptera*.—The Trichoptera have furnished some data regarding color development which are exceedingly interesting, especially as throwing light upon the coloration of Lepidoptera. In several species of Caddis flies I find that color appears first upon the head as spots, which may later fuse to form one uniform area, and then upon the thorax and ventral surface of the abdomen as spots or areas closely associated with the muscles. It is found in the wings before the imago leaves the pupa. Thus the

color develops, as a whole, much like that of the Lepidoptera. It also shows the same general features of color development as do the other orders.

*Lepidoptera*.—In this order the coloration is the most complicated and specialized found in insects; it has also been the most carefully studied. The whole surface of the body is covered by scales which in one way or another produce colors that may or may not have any relation to those produced in the body wall by the cuticula. In the larger part of the species of this order, if the scales be removed, the cuticula surface will be found to be some shade of brown due to a true cuticula pigment. This body color develops in exactly the same way as does that of the Trichoptera or Neuroptera; that is, it appears first upon the head and head appendages as spots over muscle attachments, then as similarly located spots upon the thorax and segmentally arranged spots upon the abdomen. Usually these become confluent, so that the surface has a uniform color. The coloration as it appears without the removal of the scales is often quite different from that of the cuticula and has no relation thereto. However, the segmentally arranged spots of the abdomen are sometimes directly associated with the primitive condition.

In this order, as in the Coleoptera, we must recognize two distinct colorations that are quite independent of each other. One is of ancient origin and of rather simple character, consisting of spots or stripes which have an approximately segmental arrangement and are closely associated with muscle attachments; the other is of relatively late origin and of a highly complex and special character, overlying, but not supplanting, the former, and not closely correlated with the deeper structures. This latter coloration is produced entirely by scales variously developed and modified in different species and genera.

*Homoptera*.—In this order the color patterns of the body are mainly of simple types, consisting largely of yellows, browns, and greens, with some reds and metallic colors. Many of these insects are phytophagous, and the bulk of the yellows and greens are derived from their food. The cuticula colors, as observed in *Cicada tibicen*, show close relations to the general plan of color development found in other orders. There is, however, much specialization, and many spots are developed secondarily without relation to the deeper structures. One point of interest in connection with *Cicada tibicen* is the color of the newly emerged imago, which is of a delicate pea green. As this species feeds upon the roots of plants, it has little or no chance to obtain plant chlorophyll, and the question arises as to whether this green is derived from the food or developed in the body. Only a careful study of this or allied species can solve this question.

*Hemiptera*.—The colors of this order are mostly browns, yellows, reds, and greens; the browns being cuticula colors and the reds and greens hypodermal. In *Anasa tristis* the newly hatched or newly emerged nymph at ecdysis has the head, legs, and thorax thickly set with red spots, due to lipochromes in the hypodermal cells (Pl. I, Fig. 77), but soon they become brownish by the development of cuticula

color over the parts. The abdomen shows little or no trace of the development of spots over muscle attachments, but the cuticula color first arises about numerous glandular openings, and spreads from these as centers. Upon the head and thorax much correlation exists between color areas and muscle attachments, but it is not as close or as constant as in other orders. Altogether, the species of this order which I have studied show the least of the general features of color development upon the body, its color patterns being perhaps the most specialized of any order whose diversity of coloration is not produced by scales. Certainly no other order shows so high a development of the permanent red and crimson lipochromes as the true bugs.

*Orthoptera*.—In the Orthoptera the prevailing colors are black, browns, yellows, greens, and rarely reds. The blacks, browns, and some of the yellows are cuticula colors; the greens, reds, and most of the yellows are hypodermal. The forms are largely phytophagous, and the greens and yellows are certainly in part derived from plant pigments. In ontogeny this order shows much the same type of color-pattern development as the Homoptera.

*Hymenoptera*.—The color patterns of the Hymenoptera, especially in their ontogeny, are among the most interesting to be found in insects. The colors are black, browns, and yellows, with others relatively uncommon. The patterns are largely of cuticula origin, hypodermal colors and those produced by means of scales being rare. The ontogeny of the color pattern in *Comptonotus*, *Peloporus*, *Polistes*, *Vespa*, or *Bombus* shows with beautiful clearness the development of a rather specialized color pattern from the centers of coloration over muscle attachments, as found in other orders. The colonial forms, like *Comptonotus*, *Polistes*, or *Vespa*, show admirably the stages of color-pattern development, and material is always so abundant that the stages can be followed with great detail. Among *Vespa*, *Polistes*, and *Bombus* the most specialized members show in their ontogeny stages of some duration, which are the adult condition for other species in the genus. I know of no case in insects where this is more clearly shown, unless it is in some Chrysomelid genera. This is due, however, to a lack of sharp specific differentiation in the Chrysomelidæ, and undoubtedly the same is true of the Hymenopterous genera mentioned. That is, a race or genus, as *Leptinotarsa*, *Zygogramma*, or *Diabrotica* of the Coleoptera, or *Vespa*, *Bombus*, or *Polistes* of the Hymenoptera, has spread over a large area of country, and the different conditions of habitat have modified the color pattern so that races or sub-species are formed, and these, in their ontogeny of color pattern, pass through stages which are adult conditions for neighboring races or allied species, and indicate to us much of the line of descent of these subspecies or geographical races.

In *Bombus* and some other genera the color pattern is partly formed by well-developed and often very complex scales, which usually contribute a yellow or downy appearance to the body.

*Color Patterns of Insects*

It appears from this section that insects (Pterygota) show two types of color patterns: one, as ancient in origin as the group itself, is composed entirely of cuticula and hypodermal colors, arranged approximately in segmentally placed spots and stripes, closely correlated with deeper and more vital structures; the other, of younger phylogenetic origin, is superimposed upon and obscures the older coloration; it is produced by scales, which are morphologically modified hairs of the lower insects. The first type is found in all orders of Pterygota, even though it may be modified and obscured. In development, pigmentation begins at the anterior end, appearing first over muscle attachments, or upon important sclerites, and moves posteriorly segmentally, the early stages of development being much alike in all orders, no matter how greatly the adult colors may be modified. As I have frequently mentioned, the dark colors and muscles are closely associated, and the location of spots over the muscles is not the result of chance or due to a waste product, but is, I believe, of vital physiological importance. The origin and phylogeny of these colors I shall discuss at another place. This type of color pattern permits of relatively little range of variation and but little diversity of color—facts readily noted when inspecting a large collection of Coleoptera or Neuroptera.

The second type of color pattern is younger, and not of so great physiological significance. It is pure ornamentation, and, having developed as such, shows great variability and diversity of color arrangement. Contrast, for example, the coloration of the genus *Papilio* or *Vanessa* with *Carabus* or *Diabrotica*. In the former genera the diversity is almost endless; in the latter we find series of species with browns or yellows—species after species in monotonous succession.

*Development of the Chemical Colors*

Before beginning an account of the development of colors, it will be necessary, for the sake of clearness, to consider the development of the chitinous layer with which the coloration of insects is so closely associated.

There are two opposing views concerning the development of chitinous cuticula: one regards it as produced by the modification of the distal ends of the hypodermal cells, and the other as a secretion extruded upon their surface, which accumulates and forms an even homogeneous layer and then hardens. The first view is based upon appearances found in the adult after certain kinds of fixation, and the second upon data derived from studies in embryology and metamorphosis.

At ecdysis the cuticula appears upon the surface of the hypodermis as a thin, structureless layer, perfectly continuous over the surface of the body (Pl. III, Figs. 58 and 62), there being absolutely no evidence that it is divided into blocks or that it is a modification of the end of the cell, such as has been recently described by Mercer (1900). This statement applies to material killed in Hermann, Flemming, and sublimate-acetic-acid mixtures. With Perenyi's or picric-acid mixtures, the cells are

distorted, and the delicate cuticula is poorly preserved and not differentiated from the cell below. This layer of the cuticula continues to grow by additions from below until it reaches a thickness characteristic of the species, after which no more is secreted. This is the primary cuticula from which are built, not only the outer surface of the body, but all scales, hairs, and other surface structures (Pl. III, Figs. 60, cta.<sup>1</sup>; 73 cta.<sup>1</sup>, f.).

When first formed, the primary cuticula does not react to "cuticula" stains, such as orange G or safranin, but stains intensely in thionin, iron hæmatoxylin, and other strongly nuclea or chromatic stains. What this may really mean is uncertain, but this much may be said, that the composition of the layer at this time is such that it stains much like the denser contents of the cell. While I would not in any way urge the use of a staining or any color reaction as a chemical criterion, it may tentatively be employed as indicating the general class of substances to which this first layer of chitin belongs. I shall return to this presently.

Preceding ecdysis, or the final transformation, no more cuticula is deposited, and this layer remains soft and pliable until after the larval or pupal "skin" is cast, when it begins to change rapidly. This change consists of a stiffening, which is not a process of drying, for exactly the same thing occurs in aquatic species; it is more akin to the process of coagulation, whereby the layer "sets" just as albumen sets in coagulation.

At about the time the primary cuticula begins to harden the hypodermis again becomes active, rapidly secreting a different kind of cuticula, which forms a layer of much greater thickness than the primary cuticula and gives strength to the body wall (Pl. III, Fig. 65 cta.<sup>2</sup>). This secondary cuticula is not like the first, either in structure or staining reaction. It is deposited usually in layers of alternating composition, which have different refractive indices, and give the layer the appearance of stratification. Further, it is penetrated by pore canals which run outward to the primary cuticula, but never beyond, although the ducts of the gland cells penetrate to the surface. These minute pores are thickly distributed through this layer, and are formed by protoplasmic prolongations of the hypodermal cells which reach outward to the primary cuticula (Pl. III, Figs. 61, 64, and 65, por.). These are of great importance in the development of color as well as in the final "setting" of the body wall in the adult stage.

In the larva the secondary cuticula continues to increase throughout the instar; in the adult it grows as long as nourishment is supplied to the hypodermis; after that it stops and the hypodermis degenerates. This second layer of cuticula has been found by Vosseler (1895) to give a cellulose test. This I have confirmed, but do not feel satisfied that the color reaction by which cellulose is recognized might not be used with other substances equally well and give the same result. Vosseler also recognizes in the cuticula two layers of different chemical and physical properties, such as I have shown above. This structure of the cuticula is characteristic of all the insects that have come under my observation, and, I believe, of all insects. The similarity of



structures of the cuticula is shown in the figures of sections from Hymenopterous, Lepidopterous, Coleopterous, and Hemipterous insects given on Plate III.

(a) *Chemistry of the cuticula.*—Chitin, which composes part of the cuticula of insects, is one of the most resistant of organic substances, resisting for a long time boiling in weak acids, alkalies, water, alcohol, ether, essential oils, and other organic solvents. It is easily soluble in concentrated  $\text{H}_2\text{SO}_4$  or  $\text{HCl}$ , as also in weaker reagents upon sufficiently prolonged treatment. The composition of chitin is stated variously by different workers; in all probability it will be found to be variable even in the different parts of the same individual. Thus, Sundwick gives its formula as  $\text{C}_{80}\text{H}_{100}\text{N}_8\text{O}_{38} + n(\text{H}_2\text{O})$ , where  $n=1$  to 4; Stecker (1882),  $\text{C}_{18}\text{H}_{30}\text{N}_2\text{O}_{12}$ ; Krukenberg,  $\text{C}_9\text{H}_{15}\text{NO}_6$  or  $\text{C}_{18}\text{H}_{15}\text{NO}_{12}$ , and Packard (1898),  $\text{C}_{15}\text{H}_{28}\text{N}_2\text{O}_{10}$ . It thus appears that no two workers have given chitin the same formula, which can probably be explained by the fact that no two workers have made their studies upon the same species of animal.

Chitin, according to Richter (1892), is a member of the "Chondrin group." These are nitrogenous substances, which, on boiling with  $\text{H}_2\text{O}$ , give "Gelatinates," and are either "Collogens" or "Chondrogens." In its reaction chitin is somewhat like gluten. Gluten is soluble in  $\text{H}_2\text{O}$ , and when precipitated by alcohol from its aqueous solution forms a pure, colorless, solid mass. If it be boiled with  $\text{H}_2\text{SO}_4$  or alkalies, glycol and leucine are produced, while dry distillation gives bases of the fatty and pyridin series. Alcoholic  $\text{HCl}$  converts gelatin into a compound which is further convertible by  $\text{HNO}_3$  into  $\text{C}_8\text{H}_6\text{N}_2\text{O}_3$ , which closely resembles a diazo-fatty acid, and is possibly diazo-oxyacrylic ester ( $\text{CN}_2\cdot\text{C}(\text{OH})\text{CO}_2\cdot\text{C}_2\text{H}_5$ ).

Chitin is said by some to be soluble without change in concentrated  $\text{HCl}$  or  $\text{H}_2\text{SO}_4$  when used cold, but to decompose when they are warm or boiled. Strecher (1882), however, states that chitin dissolved in  $\text{H}_2\text{SO}_4$  is changed thus:



or when boiled with alkali at  $180^\circ$  gives the following:



Chitin of lobster, when boiled with concentrated  $\text{HCl}$ , gives glycosamin ( $\text{C}_6\text{H}_{13}\text{NO}_5$ ), which is precipitated by alcohol as needle-like crystals. In the presence of  $\text{HNO}_3$ , glycosamin is oxidized to iso-sacharic acid ( $\text{C}_6\text{H}_{12}\text{O}_6$ ).

It has been shown by Strecher (1882) that a solution of chitin in cold concentrated  $\text{HCl}$  or  $\text{H}_2\text{SO}_4$ , when diluted with water, gives much grape sugar and nitrogenous decomposition products, especially  $\text{NH}_3$ , and from this he concludes that chitins are glucosides. Ledderhose (1878) also shows that a solution of chitin in cold concentrated  $\text{HCl}$  or  $\text{H}_2\text{SO}_4$ , when dropped into water at  $100^\circ\text{C}$ . and boiled, yields a substance, glycosamin, or a glucose, which reduces copper suboxide in alkaline solution. From this evidence Sundwick (1881) and Ledderhose (1878) conclude that chitin is an amido derivative of a carbohydrate having the formula  $\text{C}_6\text{H}_{100}\text{O}_{50}$ . Further, it has been

shown by Schlossberger that chitin, when kept under water for a year, partly dissolves, becomes slimy (gelatinous), and gives off a peculiar odor.

It appears thus that there are two views regarding chitin: one, sustained by Sundwick, Ledderhose, and others, that chitin is a carbohydrate derivative, as shown by the formation of glucose or related compounds; the other, that it is a nitrogenous compound belonging to the chondrogens, which are derivatives of gelatinates. Which of these two views is correct?

Vosseler (1895) shows that the chitinous integument of insects is composed of two layers which have very different chemical and physical properties, and I have shown the same thing for beetles (1900), and can affirm the correctness of this view after examining species of several orders of insects. By reference to the figures on Plate III, where sections of the integument are shown, this fact will be easily seen. On the outside there is a single homogeneous layer of material, very dense, without lamellæ or pore canals, and, when young, staining, often intensely, in "chromatin stains." Beneath this is a layer thickly pierced with pore canals, and always in layers of different refractive indices and different stainability. Vosseler found that this lower layer, or the secondary cuticula, gave a cellulose reaction, and I can affirm that with the cellulose tests it gives a reaction which strongly suggests that it is cellulose, although this is no proof. It indicates, however, that the two layers are radically different, and that the secondary cuticula is closely related to cellulose, and is, in all probability, a glucoside, perhaps tunicin.

In the preparation of chitin for analysis the custom is to boil the insect or crustacean in weak acids or alkalies *until white*, wash in water, then in weak acids, and extract. Now, the outer layer, which carries the pigment, is soluble in alkalies, or weak acids, or water upon prolonged boiling, and the result is that this layer is largely removed before the analysis is begun. Sundwick and Ledderhose prepared the chitin for their analysis in this manner and found, as Ledderhose shows, that "chitin" is decomposed by hot, concentrated HCl or H<sub>2</sub>SO<sub>4</sub>, giving a glycosamin, and Sundwick believes also a glucoside. They therefore claim that "chitin" is an amido derivative or a carbohydrate (C<sub>6</sub>H<sub>100</sub>O<sub>30</sub>), and hence glucoside, like cellulose and other plant substances.

I think it safe to conclude that we have in the integument of insects two kinds of material: one, which composes at least two-thirds of the bulk of the cuticula and has given the characteristic results to all analyses, is undoubtedly closely related to cellulose and is a glucoside, containing some nitrogen substances mixed with it; the second layer is thin, composing less than one-third the bulk of the chitin, and is of a chemical nature quite different from the inner, or secondary, cuticula. What is the nature of this outer, or primary, cuticula?

In development, this layer is the first to be laid down; it is soft, pliable, homogeneous in character, and stains intensely in chromatin stains. As development proceeds it becomes more or less pigmented, and eventually forms the hard and brittle

outer surface of the body. When first formed, or at ecdysis, this layer is soluble in pure water. After having once hardened, however, it is not easily soluble, although prolonged subjection to water, weak acids or alkalies, but more especially water, partly dissolves this layer and gives a gelatinous surface or residue, if the solution be carried far enough. The secondary cuticula is less affected by this treatment. In such solutions, besides nitrogenous waste products,  $\text{NH}_3$  is found to some extent. The facts that the primary cuticula is soluble, or partly so, in water at an early stage of development, and that later it is still soluble, giving a gelatinous residue and nitrogenous waste products, together with its homogeneous character and stainability, lead one to conclude that this layer of the integument should undoubtedly be classed as a derivative of some gelatinates which are probably, as Richter believes, closely related to one of the "Chondrin group," if not actually one of them. It is this layer that carries the coloring material which gives the important and widely disseminated cuticula colors.

(b) *Development of the cuticula colors.*—Cuticula colors are generally recognized to be the most stable of any found in insects, and to a high degree insoluble by the usual means. They were called by Poulton true pigments, or colors proper to the species. Coste, Urech, Mayer, and others have noted that the blacks, browns, and many yellows are very stable, and I have pointed out that these colors are usually, if not always, of cuticula origin.

I find that these colors are not readily soluble in any reagent without dissolution of the cuticula. Thus, solutions may be prepared by hot or cold concentrated  $\text{HCl}$ ,  $\text{H}_2\text{SO}_4$ ,  $\text{HNO}_3$ , aqua regia, strong alkalies, etc., but the cuticula is dissolved as well as the pigment. Prolonged treatment with water or alcohol dissolves the color in part and reduces the cuticula to a slimy or gelatinous consistency. Ether, essential oils, and other organic solvents are quite ineffectual. It thus appears that these colors are very intimately associated with the primary cuticula and are, as will appear later in this section, derived from it, if they are not cuticula itself. With solutions of this cuticula color various tests can be made, but their value and accuracy is, in my experience, entirely unreliable. It is shown conclusively that when cuticula is dissolved in the concentrated inorganic acid, which forms the most convenient solvent, there is a change in the composition of the chitin and by-products; substances of simpler composition are produced, and the testing of solutions like these for pigments, etc., has, I believe, very little value.

The study of animal and plant pigments has been carried on almost entirely by means of spectrum analysis, by far the larger part of the pigments of organisms having been recognized by their so-called spectra and named after the animal in which they are found. There is, however, very grave doubt as to the value of such spectrum work. After spending several months in the analysis of cuticula pigments of Coleoptera, Lepidopterous larvæ, and Hymenoptera, I discarded as worthless all of my results, because in no case was I able to obtain a spectrum of sufficient perma-

nence, or one that had absorption bands of enough constancy, to be reliable. The same solution with the same apparatus and light will vary in its spectrum from day to day. Moreover, any slight change in acidity or alkalinity, or in dilution or concentration, etc., will produce a variation in the spectrum. If, however, the above difficulties can be overcome and a constant spectrum be obtained, of how much value is this? Can it be used to indicate chemical relationship, or even relationships of organic pigments?

A concentrated HCl solution of the cuticula of *Parsallus cornutus* is deep brown in color and gives a fairly constant spectrum, but a solution of india ink, Vandyke brown, or sepia gives a spectrum which, in proper dilution or concentration, cannot be distinguished from that of *P. cornutus*. No one would attempt to group these substances as related compounds or pigments. It has been shown also by Newbegin (1898) that hæmoglobin, carmine, and turacin produce identical spectra, yet these are known to have different compositions and to be in no wise related to one another.

If the spectrum were of any value in recognizing organic coloring substances, one would expect to find it used in the chemistry of analin. As a matter of fact, chemists have not used it for the reason that its results are entirely unreliable. The chemist uses spectra rarely or not at all to identify compounds, and only habitually to recognize those elements which have very definitely located and unmistakable absorption bands. If, then, in compounds whose composition and affinities are known, the spectrum test fails as a means of identification and classification, how utterly valueless must be its use with unknown compounds! I fully agree with Newbegin (1898) that data concerning pigments drawn from spectrum analysis are valueless, or nearly so, and I believe that pigments so determined cannot again be certainly recognized.

It is frequently assumed by writers upon the subject of coloration in animals that the coloring matter is a waste product removed from the body by one cause or another and deposited near the surface. This view has been discussed by Urech, Newbegin, and others, and need not be repeated here. But why should this deposition be in a pattern that is constant for each species? Indeed, the best evidence shows quite conclusively that the pigments of animals are not in most cases waste products, and cuticula pigments are most assuredly not, as will appear in the discussion of their cause.

Many hypodermal and sub-hypodermal pigments of animals, as the greens and yellows of larvæ, seem to be derived from their food and are not at all permanent. Cuticula pigments, however, are not derived, but are, as Poulton says, "proper to the species," or "true pigments."

In an earlier paper (1900) I advanced the view, supported by some evidence, that the cuticula colors are due to secretions poured out upon the surface of the cuticula. This assertion was based partly upon the existence of deeply staining granules in the pore canals of the cuticula which were derived from the hypodermal cells; and, further, upon the appearance of the primary cuticula, which seemed to be in blocks or

masses upon the surface of the secondary cuticula. The material used at that time was killed in Perenyi's fluid or picro-sulphuric acid, which is now known to be an unreliable preservative for these structures. A careful study of Coleoptera, as well as other orders, has shown the error of my former interpretation, for the following reasons: (1) the primary cuticula, wherein lie the cuticula pigments, is first laid down as an even, homogeneous layer over the outer surface, which is pierced, not by pore canals, but by the ducts of the hypodermal glands; (2) the granules observed in the pore canals are now known not to be secreted pigment, but zymogens, a material of a very different character. These facts, along with many others, have made untenable my former interpretation that the cuticula pigments are secreted pigments.

It is frequently asserted, but not upon any authority that I can find, that the pigments of insects (the cuticula colors) are due to the drying of the cuticula. It is merely assumed that this is so. Drying of the cuticula would hardly account for the existence of these colors in aquatic insects, or in species that pupate in damp places. If dryness is productive of these pigments, we might expect to find them entirely absent in aquatic forms and abundant in those of deserts; but the facts in nature do not warrant this conclusion, and the same is easily demonstrable in experiment.

Another assertion one frequently finds is that contact with the oxygen of the air produces coloration. This is also, so far as I can find, pure assumption. If this were the cause, we should expect increased or decreased amounts of oxygen to accelerate or retard the formation of colors. To test this hypothesis I subjected pupæ to atmospheres containing varying amounts of oxygen, as follows: pupæ of *L. decemlineata* were kept during the pupal stage and that of the adolescent imago in atmospheres consisting of O<sub>2</sub> 40 pts., N 80 pts.; O<sub>2</sub> 60 pts., N. 80 pts.; O<sub>2</sub> 80 pts., N 80 pts.; and O<sub>2</sub> pure. In the first two no changes were noted, but in the third there was a large mortality, showing that the amount of O<sub>2</sub> had become toxic, and the pigmented areas were small and weak. The same results were attained in a more marked manner with pure O<sub>2</sub>.

A second set of experiments consisted in diminishing the O<sub>2</sub> present; but even though O<sub>2</sub> was absent, pigmentation was not changed. A third set consisted in placing pupæ in an atmosphere of CO<sub>2</sub>, N, H, and pigmentation, if about to begin or already begun before the pupæ were placed in the gas, was not retarded or changed. From these results we may safely conclude that if the oxygen of the air were at all responsible for the cuticula colors, there would have been in these experiments an acceleration or retardation of pigmentation.

It thus appears that the cuticula colors are not due to derived pigments, to secretions, to drying, to atmospheric oxygen, or to oxidation. What, then, is their cause? After discarding all these explanations I began experiments of various kinds for the purpose of finding a clue to the cause, and after much profitless work observed that, if pieces of the integument which had been removed from the body of a beetle or wasp just before pigmentation began were kept from drying, they developed their pigment

to a greater or less extent, and that this pigment was normal. I further found that color develops in pupæ for some time after death, and that in a vapor of chloroform color development is not seriously retarded, if at all. These observations, especially the last one, suggested the possibility of the existence of some katalytic agent or enzyme as a factor in pigmentation, or perhaps its cause.

Experiments now followed to determine whether the presence and action of enzymes could be shown more clearly. These grouped themselves under three heads: (1) experiments with pupæ or fragments thereof for the study of the retardation of pigmentation and for the production of post-mortem coloration; (2) experiments with extracts of the enzymes to determine their action upon unpigmented cuticula; (3) microscopic examination of tissue to determine, if possible, the existence of zymogens and the histological and cytological processes involved.

It seems a well-established fact that many enzymes are able to carry on their work under conditions which inhibit the ordinary life-processes, if they do not actually destroy them. Thus, vapor of chloroform, while it stops the action of bacteria or other organisms, does not check the action of enzymes secreted by an organism. For the purpose of determining as fully as possible what effect vapor of chloroform has upon the development of the color pattern, pupæ of *Chrysobothris femorata* and *Leptinotarsa decemlineata* were used, as were also pupæ of some other Coleoptera and Lepidoptera which need not be mentioned here.

Some time before any pigmentation had begun, pupæ were placed in vessels containing vapor of chloroform, either pure or diluted with air. Plenty of moisture was supplied, and they were kept at the proper temperature. Pupæ placed in chloroform vapor just after pupation, or at any time preceding a period of about twelve hours before the beginning of pigmentation, did not develop any color. Those placed in chloroform vapor just before pigmentation began usually developed the color pattern partially, but never completely; but if they were allowed to begin the development of pigment before being placed in the vapor, the color pattern was completed as far as it is normally in the pupa. We learn from these experiments that chloroform vapor checks pigmentation if applied before a certain time in the life of the pupa, and that, if applied after the process has begun, pupal pigmentation is not to any great extent interfered with. Dilute alcohol gave a result somewhat similar when used at from 10 to 30 per cent. strength.

Temperatures of varying degrees also gave interesting results. Thus, *C. femorata* did not seem to be able to survive a temperature above 40° C., yet, when kept at 45°, if not placed in the bath previous to some six to twelve hours before pigmentation began, the color would develop, although somewhat erratically. With *L. decemlineata* I have frequently obtained this same result in high-temperature experiments. The color pattern on the head and pronotum would develop, not only after death, but also after much post-mortem change had taken place. With low temperatures, however, if low enough, —5° C., the color-pattern development was seriously inhibited.

These experiments serve to show that coloration, when about to begin or already begun, is not checked by those agencies which inhibit or destroy the vital process, so-called. If, however, the inhibitory stimulus is applied before the color is about to appear, coloration development is effectually checked. The reason for this will appear, I believe, when we examine the tissues for evidence as to the existence and development of zymogens.

If enzymes are at work in the production of coloration, we ought to be able to extract these substances from the tissues, and with them obtain a reaction upon the cuticula similar to that which they produce normally. Considerable difficulty attends this operation on account of the necessity of opening the pupa in distilled water, of carefully separating the body wall from the rest of the animal, and of washing and extracting; furthermore, many individuals must thus be prepared in order to get any results. Pupæ of *C. femorata* so prepared were ground with pure quartz sand and the enzyme extracted, first with pure water, then with dilute alcohol, dilute alcohol and acetic acid, and dilute alcohol and glycerine. Dilute alcohol and acetic acid served the best, as it gave the largest amount of precipitate upon the addition of 95 per cent. alcohol. The white, rather flocculent precipitate produced was separated by filtration, redissolved in alcohol and acetic acid, and reprecipitated several times, and finally used in 30 per cent. alcohol containing  $\frac{1}{10}$  per cent. acetic acid.

In the extract thus obtained were suspended pieces of the primary cuticula taken from young pupæ before the secondary cuticula had begun to develop. All adhering hypodermis was removed, and the operation was performed in the presence of chloroform vapor. The result was that in about thirty-six hours the cuticula had become brown, being first drab, then pale brown, and finally a full brown. Beyond this coloration did not advance. It was uniform over the entire surface, but, although areas where spots normally develop were present, no tendency to spot formation was observed. When this solution was used with cuticula from other species, only faint coloration was produced, or none at all.

Other species of Coleoptera were also tried, but it was difficult to obtain a sufficient amount of material to make the extraction of these color-producing substances perfectly certain. From the pupæ of *Polistes*, which can be obtained in considerable numbers, a substance was extracted by the same method which upon the cuticula of *Polistes* produces like results.

In plants and in vertebrates the production of enzymes may take place in two ways: definite granules of zymogen which have formed and gathered in the inner zone of the cells, as in the case of vertebrate glands, are extruded and transformed into enzyme on the outside; or the enzyme may exist diffused through the protoplasm of the cell, giving to the protoplasm a katalytic action.

It was impossible to prove the existence of a zymogen in the hypodermis of much of my material, as almost all the best of it was in sublimate-acetic-acid fixation, which does not fix zymogens well, but dissolves them and leaves vacuoles where the

granules should have been. The best results were obtained, and the cell was also fairly well preserved, in material fixed in 80 per cent. alcohol at 70–80° C. Weaker grades also gave fair results. With Perenyi and picric-acid mixtures some zymogen granules were preserved, but the rest of the material was so bad that the preparations were useless. The account of the zymogens and their part in pigmentation is based upon material from *L. decemlineata* and *C. femorata*. For demonstrating the presence of substances, probably zymogens, I used iron hæmatoxylin, which is fairly good, and Bensley's stain for zymogens.

In the pupa of *C. femorata*, fixed in sublimate acetic acid about five days after pupation, I found what I believe to be the first trace of the formation of secreted material within the cell. At this time the primary cuticula is just forming, and the cytoplasm of the hypodermal cells is dense and granular; the nucleus, which is small, has a dense chromatin reticulum with frequent denser aggregations all closely applied to the membrane of the nucleus. Evidence for the existence of a secretion is shown by the presence of an empty vacuole, or series of vacuoles, lying near the nucleus (Pl. III, Fig. 58, vac.). This feature is uniform and is due, I believe, to the removal of the contents by the reagents employed.

The next stage found is shown in Fig. 59, Pl. III, taken about three hours before pigmentation began. Here were found in the cytoplasm of the cell large globules of a homogeneous material which gathered rarely into large drops or aggregations just below the cuticula, as shown in Fig. 59. The hypodermal cell has increased in size, the nucleus has become very large, and the chromatin network much reduced and dispersed. A later stage in which the color has attained about half its mature condition is shown in Fig. 60, Pl. III. Here the amount of secreted material has become much less, vacuoles exist in the cytoplasm where the globules of secretion were, and the nucleus shows a further reduction of chromatin and a less dense stain.

At about this time the secondary cuticula begins to be deposited. Before this begins, however, the globules of secreted material have disappeared from the cytoplasm, leaving only traces in the vacuoles. The adult condition of the body wall is shown in Fig. 61, Pl. III, where the primary cuticula is deep brown, and the secondary cuticula well developed and pierced by numerous pore canals. The hypodermal cells have become dense and granular, the nucleus stains hardly more than the cytoplasm, and the chromatin matter is evidently diffuse and poorly defined.

In *L. decemlineata* similar but more complicated conditions are found. In an early stage (Pl. III, Fig. 62) of the pupa the hypodermal cells are found to have gathered about the nucleus numerous highly refractive globules, which stain in Bensley's stain for zymogens. In sublimate acetic material these are absent and vacuoles are found in their place, which shows that the substance is removed by certain reagents and precipitated by strong alcohol. These granules are found at about the time the primary cuticula reaches its completion. Immediately after this the secondary cuticula begins to be formed (Pl. III, Fig. 63), and there are found numerous pore



canals which pass upward from the hypodermis to the primary cuticula (Pl. III, Fig. 64); it is along these that the secreted granules pass to the primary cuticula when pigmentation begins.

The development of the color differs in no essential manner from that of the preceding species. The granules of secreted material, which are here small, migrate outward along the pore canals (Pl. III, Figs. 64 and 65) and then break down. Eventually these bodies all disappear from the cells and the cuticula attains its adult color. The changes in the cytoplasm and nucleus are like those of the preceding species. (Compare Pl. III, Figs. 61 and 66.)

It thus appears that secretions in the form of globules are formed in the hypodermal cells accompanied by a chromatolysis of the nucleus, and that these secreted bodies come to lie at the proximal side of the primary cuticula, and are there lost as definite bodies and do not stain. Soon afterward color appears in the cuticula, and slowly increases in intensity. These secreted bodies are themselves colorless and stain intensely in iron hæmatoxylin and Bensley's zymogen stain. It further appears that these bodies are soluble in many reagents, but are precipitated by strong alcohol. In every way they react and develop as do zymogen granules in the glands of vertebrates.

It further appears that there can be extracted from the integument, best by dilute alcohol and acetic acid, a substance which produces color in cuticula taken from animals before their bodies have come in contact with it. It is also found that the colors will develop in the presence of substances which inhibit the life-processes.

In view of these facts I think it safe to conclude that there is formed by the hypodermis some katalytic agent or enzyme which, passing out through the pore canals, comes in contact with the primary cuticula and there becomes the active factor in the production of cuticula colors. Further evidence that some such agent is at work is furnished by the condition of the cuticula before, during, and after pigmentation.

The cuticula of insects is colorless only at the joints, where it remains soft and pliable. Wherever it becomes hard and resistant, a yellowish, brown, or black color is developed. The way in which a section of the cuticula cuts is a good indication of its quality and age. Before pigmentation begins it is soft, easily permeable to imbedding reagents, and gives perfect sections. As color appears, however, there is experienced a difficulty in sectioning and infiltration, which increases as the coloration develops.

I have previously shown that this hardening of the cuticula is not due to drying, oxidation, or other causes to which it is commonly ascribed; the only tenable view is that some agency produces a setting or clotting of the soft, plastic cuticula; or, in other words, that the process is due to a katalytic agent or enzyme. Evidence that an enzyme is present and active is found: (1) in the formation of zymogen in the hypodermal cells, the arrangement of the secondary cuticula to allow this to come in contact with the primary cuticula, and the migration of the granules to the primary cuticula, where they break down; (2) this product of the decomposition of the zymogen is

extractable in alcohol and other enzyme solvents, and in alcoholic solution produces exactly the normal effect upon fresh, soft cuticula; and (3) substances which inhibit vital processes but allow enzymes to work, also allow of the hardening of the cuticula and the development of color. Hence, there can be no doubt that an enzyme or enzymes are at work in this process. It is evident that the enzymes are related to the soluble ferments of proteins, some of which, through the setting or coagulation of albuminoid and gelatinous substances, produce proteoses, peptones, and amido compounds. For these enzymes, which will certainly prove to be numerous, I propose the name "chitases."

(c) *Nature and composition of cuticula colors.*—The chemical nature of colors is a problem most difficult of investigation, chiefly because such energetic measures are necessary to get the color into solution that there is every reason to suspect that it is no longer the same as that in the cuticula. With solutions of the cuticula color of *P. cornutus* in HCl or H<sub>2</sub>SO<sub>4</sub>, various tests were made, which established the fact that it is much like some of the benzene derivatives, and the spectra of this solution and of permanent brown are identical and closely similar to that of Bismarck brown. There is, however, a great difference between the solution of cuticula color and Bismarck brown, the former being a *colored substance* and the latter a *coloring substance*.

Cuticula colors in acid solution are decolorized by reducing agents such as tin and HCl or strong alkalis. If, however, the decolorized solution is treated with a mild oxidizing agent, part or perhaps all of the color is restored. In the process of decolorization the solution passes from deep brown to lighter shades, to yellow, and eventually to a colorless condition; oxidizing agents produce the reverse changes on the colorless solution. According to Stecher and others, fast brown is a diazo compound belonging to the group of amidoazo and oxyazo compounds, which form colored solids varying from yellow to deep brown. These diazo, oxyazo, and amidoazo compounds are soluble in alcohol, as is this cuticula color, and when in solution are rendered colorless by reducing agents, thus forming colorless azo or hydrazo compounds, which, by mild oxidation, may be reconverted into yellow- or brown-colored diazo, oxyazo, or amidoazo compounds. There is thus a very close agreement between the reaction of cuticula color and the diazo, oxyazo, and amidoazo compounds to reducing and oxidizing agents. In these and other characters the cuticula colors resemble these benzene derivatives, but not in any respect do they resemble other colored substances or dyes known to organic chemistry. The existence of azo compounds has been recognized by Bottler (1902) in the hairs of animals and in silk fibers, where they function as pigments and have some of the structural peculiarities of cuticula color. I feel perfectly certain, therefore, that these cuticula colors are azo compounds, a conclusion based upon the following characters: (1) their colors; (2) solubility; (3) behavior to oxidizing and reducing agents; (4) crystallization; (5) spectrum; and (6) their decomposition products.

The primary cuticula, when first deposited, is, as has been shown, a soft, gelatinous

and highly complex albuminous body which, by the action of an enzyme, is converted from a complex, unstable state to one very stable. In this there are undoubtedly produced two classes of substances: one, chitin, which hardens and forms a tough shell or covering to the animal; the other, an amido compound which, by further modification, becomes amidoazo, diazo, or oxyazo compounds—colored substances that are included in the layer of primary cuticula as it hardens.

There is every reason to believe that chitase is a proteolytic enzyme which acts upon a freshly formed, complex, albumino-gelatinate, commonly called chitin, but which, for convenience, I shall distinguish as pro-chitin, converting it into an amido compound series and true chitin—a reaction which is comparable to that of many proteolytic enzymes. This action of the enzyme or enzymes present, as I have pointed out before, varies in different parts of the same segment. Thus, between two contiguous segments or between movable sclerites the chitin always remains soft, pliable, and unpigmented, easily permeable by reagents and easily sectioned; that is, it remains throughout life in the original condition of the whole chitinous layer. The sclerites composing the segments, especially those to which muscles are attached, as already shown, become hard and form an exoskeleton, and in this hardening they all become colored more or less, the colors ranging from yellow to deep brown. The yellow sclerites are softer and cut more easily than the deep brown or black ones, the hardness of the sclerite and the amount of color being in direct proportion. Hence it seems altogether probable that the continued action of the enzymes is productive of hardness and color. In this sense the color is a by-product of the development of a hard outer covering or exoskeleton, but it is not in the usual sense of the term a waste product.

In the preceding pages I have sketched briefly my conclusions concerning the development and composition of the cuticula color of insects, so far as I am convinced of their correctness. In a future paper I shall present in detail my data and experiments, and I hope to be able in addition to state more exactly the composition of colors, and the characters of the agents which produce them. In this paper I have dealt entirely with cuticula pigments and color patterns. Many data, however, concerning the hypodermal colors and their patterns have already been obtained and will appear elsewhere.

#### *Colors and Color Patterns*

In preceding sections I have shown that the most important and widely disseminated of insect colors are those of the cuticula. From the last section it appears that these colors are not due to drying, oxidation, secretion, or like processes, but are produced by katalytic agents working in the cuticula. Further, these colors develop as the cuticula hardens, and appear first, as a rule, upon sclerites to which muscles are attached. In one of the earlier sections of this paper I showed that the pigment develops from before backward and, approximately, by segments, excepting that it may appear upon the head and most posterior segments simultaneously.

In ontogeny color appears first, as a rule, over the muscles which become active first, or upon certain sclerites of the body. These are usually the head muscles, although exceptions are not infrequent. It should be remembered that as the color appears the cuticula hardens, and, considering that muscles must have fixed ends for their action, it seems that there is a definite relation between the development of color, the hardening of the cuticula, and the beginning of muscular activity; the last being dependent upon the second, and, incidentally, accompanied by the first. As muscular activity spreads over the animal the cuticula hardens and color appears, so that color is nearly, if not wholly, segmentally developed.

The relation which exists between cuticula color and the stiffening of the cuticula is thus a physiological one, the cuticula not being able to harden without becoming yellow or brown. What bearing has this upon the origin of color patterns? In the lower forms of tracheates, such as the Myriapods, colors appear as segmental repetitions of spots or pigmented areas which mark either important sclerites or muscle attachments. On the abdomens of insects, where segmentation is best observed, color appears as well-defined, segmentally arranged spots, but on the thorax segmentation is obscured and lost upon the head. Of what importance, then, is pigmentation? And how did it arise? If the ontogenetic stages offer any basis for phylogenetic generalization, we may conclude that cuticula color originated in connection with the hardening of the integument of the ancestral tracheates as necessary to the muscular activity of terrestrial life. The primitive colors were yellows, browns, and blacks, corresponding well with the surroundings in which the first terrestrial insects are supposed to have lived. The color pattern was a segmental one, showing repetition of the same spots upon successive segments, as upon the abdomen of Coleoptera.

So firmly have these characters become ingrained in the tracheate series, and so important is this relation of the hardening of the cuticula to the musculature and to the formation of body sclerites, that even the most specialized forms show this primitive system of coloration; and, although there may be spots and markings which have no connection with it, still the chief color areas are thus closely associated.

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## EXPLANATION OF PLATES

The optical parts used were, unless otherwise stated, Zeiss apochromatic objectives, 2, 3, and 4 mm. and compensating oculars. All figures were outlined with Abbé camera and detail put in free hand.

## ABBREVIATIONS USED

cta.	cuticula.	h'drm.	hypodermis.
cta. <sup>1</sup>	primary cuticula or pigmented cuticula.	l'p'chr.	lipochrome.
cta. <sup>2</sup>	secondary cuticula.	nebr. pr.	basement membrane.
cty.	cytoplasm.	por.	pore.
cor. ad.	fat body.	lam.	lamella.
cl. for.	formative cell.	sq.	scale.
dc.	duct.	vac.	vacuole.
fbr.	fiber.	zyg.	zymogen granule.
gl.	gland.	nuc.	nucleus.
hae'ly.	hæmolymph.		

## PLATE I

FIGS. 1-11. *Orthosoma brunneum* Forst. From Cold Spring Harbor, L. I. A series of pupæ and young imagines showing the ontogeny of the color pattern. Drawn from sketches and notes taken from the living beetles.  $\times 1/1$ .

FIGS. 12-21. *Leptinotarsa decemlineata* Say. A series of pupæ and larvæ showing the general features of the ontogeny of the color pattern. The pupæ are supposed to be straightened out.  $\times 2/1$ .

## PLATE II

FIGS. 23-28. *Orthosoma brunneum*. A series of stages showing the detail of color-pattern development upon the pronotum.  $\times 4$ .

FIGS. 29-34. *Chrysobothris femorata*. A series of stages showing the detail of color-pattern ontogeny upon the pronotum.  $\times 5$ .

FIGS. 35-42. *Leptinotarsa decemlineata*. A series of stages showing the detail of color-pattern development upon the pronotum.  $\times 5$ .

FIGS. 43-45. *Orthosoma brunneum*. Three stages in the development of color upon the ventral abdominal surface.  $\times 2/1$ .

FIGS. 46-50. *Phymalodes* —. A series showing development of color pattern upon the elytra.  $\times 5$ .

FIGS. 51-53. *Pelidonata punctata*. A series showing development of cuticula colors.  $\times 4$ .

## PLATE III

FIGS. 54-57. *Chrysobothris femorata* Fab. Four stages in the development of color in the elytra, showing development of cuticula color first and then the green metallic color.  $\times 2/1$ .

FIG. 58. *Chrysobothris femorata*. Section of the dorsal layer of hypodermis in an elytron of a pupa showing the first beginnings of the primary cuticula (cta.<sup>1</sup>), the nucleus with a dense chromatin network, and the dense granular cytoplasm. Sublimate-acetic-acid preparation, and iron hæmatoxylin stain. Zeiss 4 mm. obj., No. 12 oc., 160 mm.

FIG. 59. *Chrysobothris femorata*. Section of the dorsal layer of the hypodermis of an elytron in an imago just after the expansion of the wings and before any color appears, the wings being white. The primary cuticula is fully developed, but is transparent, and in the hypodermal cells are large globules of a material that stains with iron hæmatoxylin, thionin, or other chromatin stains. Hot 70 per cent. alcohol and thionin. Zeiss 2 mm. obj., No. 12 oc., 160 mm.

FIG. 60. *Chrysobothris femorata*. Section same as in Fig. 61, but later corresponding to Fig. 25, or at the first appearance of metallic color. At this stage the primary cuticula is brownish, and its outer surface has developed two lamellæ. Fixation and stain as in Fig. 60. Zeiss 3 mm. obj., No. 18 oc., 160 mm.

FIG. 61. *Chrysobothris femorata*. Section of the dorsal lamella of the mature elytron near the costal edge, showing completely blackened primary cuticula (cta.<sup>1</sup>), the secondary cuticula (cta.<sup>2</sup>) traversed by pores (por.), and the exhausted hypodermal cells (h'drm.) with the nearly achromatic nuclei. Zeiss 2 mm. obj., No. 9 oc., 160 mm. Sublimate acetic acid. Delafield.

FIG. 62. *Leptinotarsa decemlineata*. Section of the dorsal lamella of an elytron in the pupa, showing the primary cuticula (cta.<sup>1</sup>) and the hypodermal cells containing many highly refractive granules (zyg.). From preparation killed in 85 per cent. alcohol at 80° C. and alum carmine. Zeiss 2 mm. obj., No. 8 oc., 160 mm.

FIG. 63. *Leptinotarsa decemlineata*. Section of an elytron through the dorsal lamella, just after the wing has expanded and before color begins to appear. From a sublimate-acetic-acid preparation, hæmalum. Zeiss 3 mm. obj., No. 6 oc., 160 mm.

FIG. 64. *Leptinotarsa decemlineata*. Section of an elytron through the dorsal lamella at the beginning of pigmentation in a "yellow" area showing primary cuticula (cta.<sup>1</sup>) just beginning to become yellow, the secondary cuticula traversed by pore canals (por.) in which granules (zyg.) are seen which have come from the hypodermal cells. Preparation killed in 85 per cent. alcohol at 80° C., stained in Bensley's stain for zymogens. Zeiss 2 mm. obj., No. 12 oc., 160 mm.

FIG. 65. *Leptinotarsa decemlineata*. Section from same preparation as Fig. 65, but through an area destined to become black. Zeiss 2 mm. obj., No. 12 oc., 160 mm.

FIG. 66. *Leptinotarsa decemlineata*. Section through an elytron in a dark area. Sublimate acetic acid, hæmalum. Zeiss 2 mm. obj., No. 12 oc., 160 mm.

FIG. 67. *Leptinotarsa decemlineata*. Section from the same preparation as Fig. 67, but through a yellow area. Zeiss 2 mm. obj., No. 9 oc., 160 mm.

FIG. 68. *Orthosoma brunneum*. Section of an elytron in the "yellow-brown" stage, corresponding to Fig. 5, showing the primary cuticula (cta.<sup>1</sup>) beginning to become colored in the inner portion. Preparation, alcohol 80 per cent. at 70° C., iron hæmatoxylin. Zeiss 3 mm. obj., No. 12 oc., 160 mm.

FIG. 69. *Orthosoma brunneum*. Section of a mature elytron. Preparation as in Fig. 68. Zeiss 3 mm. obj., No. 12 oc., 160 mm.

FIG. 70. *Clisiocampa americana*. Section of the integument of the larva just before the beginning of the prepupal stage, showing the primary cuticula (cta.<sup>1</sup>) pigmented and on its outer surface thickly studded with conical processes. The secondary cuticula (cta.<sup>2</sup>) is thick, longitudinally striated, and pierced with numerous pore canals. In the hypodermis are seen numerous undissolved and unstained granules of a lipochrome (?) pigment (l'p'chr.). Preparation, sublimate acetic acid, Mayers hæmalum. Zeiss 4 mm. obj., No. 12 oc., 160 mm.

FIG. 71. *Clisiocampa americana*. Section of the body wall of a larva in the third instar through a dorsal red spot, showing transparent place in the pigmented primary cuticula and the red lipochrome pigment below. Preparation and magnification as in Fig. 72.



FIG. 72. *Polistes pallipes*. Section through the body wall of the abdomen, showing primary cuticula becoming colored (cta.<sup>2</sup>), the striated secondary cuticula (cta.<sup>3</sup>) pierced by the canals of the formative cells (cl. for.) of the hairs or scales (sq.). Preparation, picro-sulphuric acid, alum carmine. Zeiss 4 mm. obj., No. 18 oc., 160 mm.

FIG. 73. *Polistes pallipes*. Section of adult through the same region as Fig. 75. Preparation and magnification as in the preceding figure.

FIG. 74. *Polistes pallipes*. Section of adult yellow area, showing the yellow cuticula color. Preparation, Perenyi, Delafield. Zeiss 3 mm. obj., No. 12 oc., 160 mm.

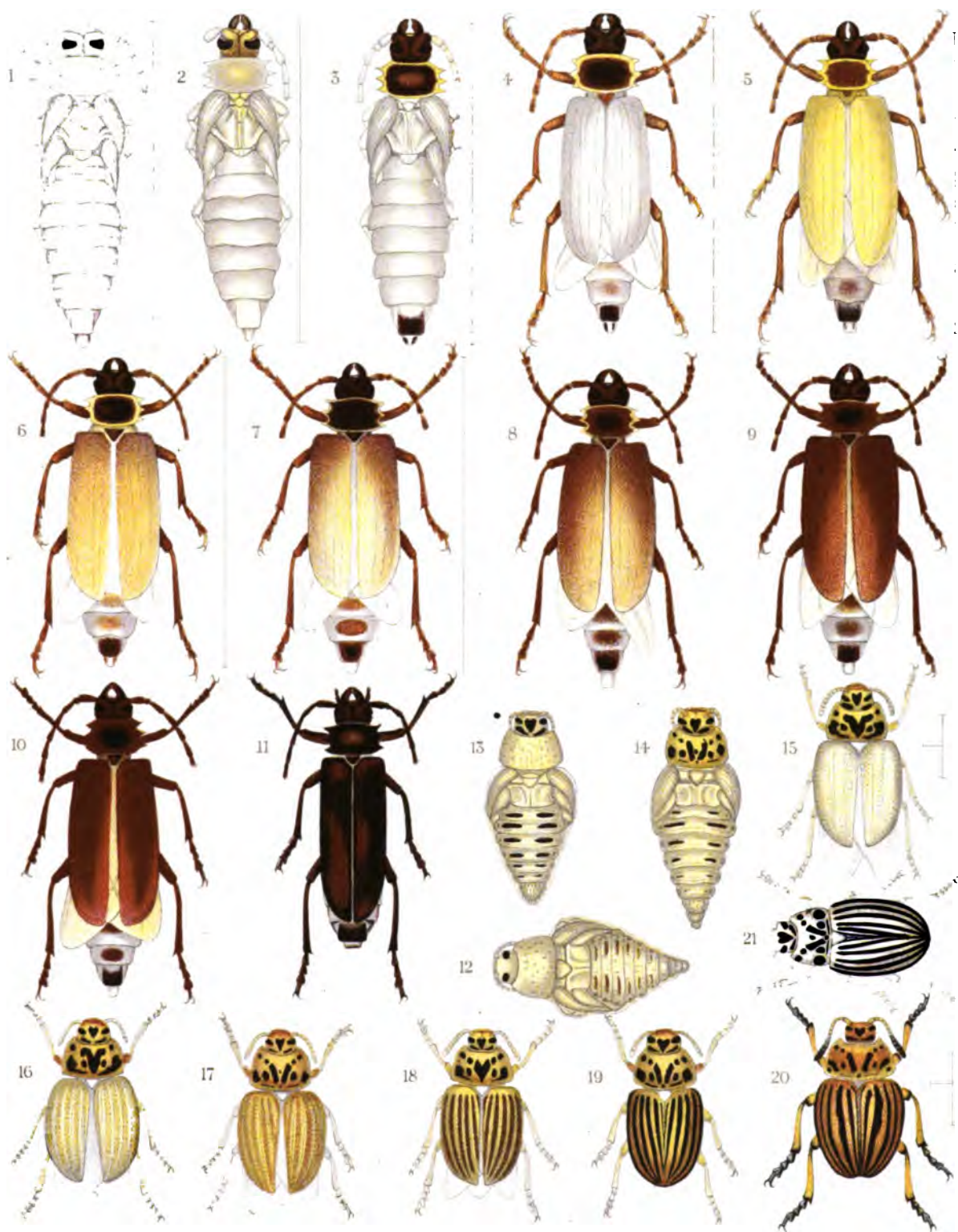
FIG. 75. *Epilachua borealis*. Frozen section of the larval body wall showing the transparent primary cuticula (cta.<sup>1</sup>), the hypodermis, and hæmolymph highly colored by a yellow pigment, probably xyanthrophyll, derived from the food. Bausch and Lamb  $\frac{1}{4}$  inch obj.,  $\frac{1}{4}$  inch oc., 180 mm.

FIG. 76. *Coccinella bipunctata*. Frozen section of the larval body wall through a yellow spot showing color produced by the pigment head in solution in the hæmolymph. Bausch and Lamb,  $\frac{1}{8}$  inch obj.,  $\frac{1}{2}$  inch oc., 160 mm.

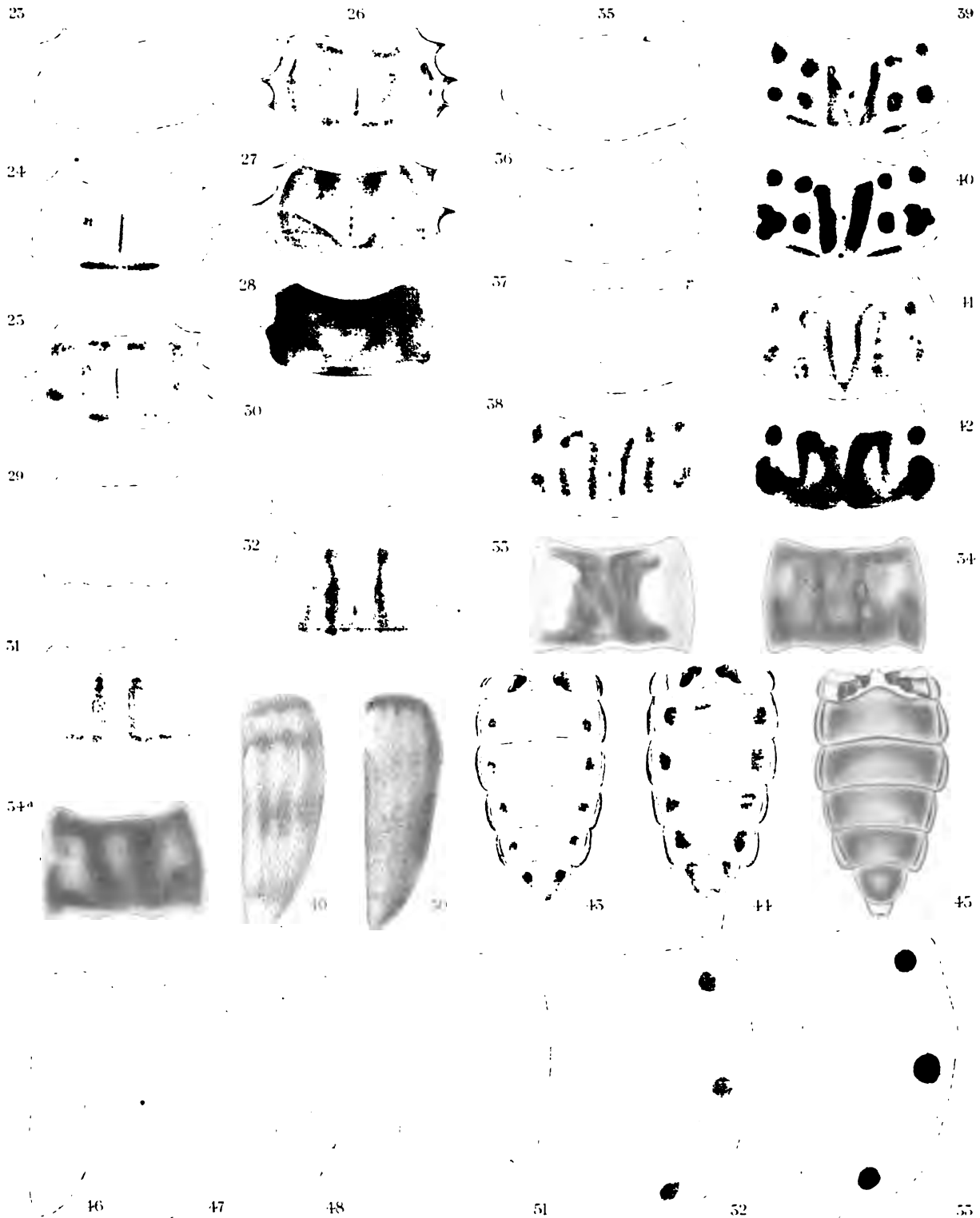
FIG. 77. *Anasa tristis*. Section through the metathorax of a nymph just hatched, showing the transparent primary cuticula (cta.<sup>1</sup>), the aggregation of granules of red lipochrome pigment in the hypodermis forming the red spots. Preparation, sublimate acetic acid, Mayer's hæmalum. Zeiss 3 mm. obj., No. 12 oc., 160 mm.

FIG. 78. *Anasa tristis*. Section through the metathorax in the second instar, showing coloration of the cuticula and the consequent dulling of the red color. Preparation as in Fig. 70. Zeiss 2 mm. obj., No. 18 oc., 160 mm.

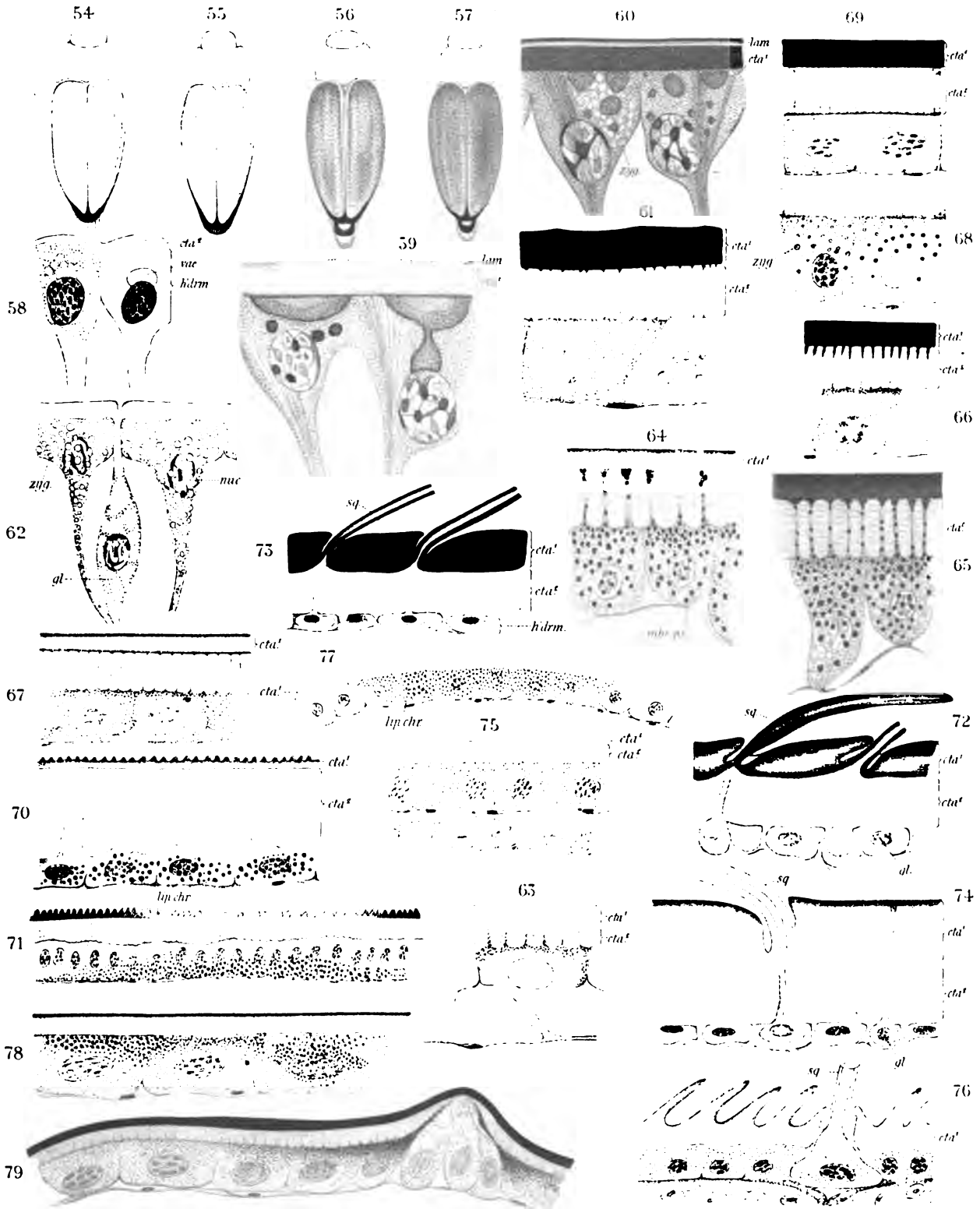
FIG. 79. *Anasa tristis*. Section through the mesothorax of a nymph of the fourth instar, showing the complete obscuring of the red color by the cuticula browns. Preparation as in Fig. 70. Zeiss 2 mm. obj., No. 18 oc., 160 mm.













## **ARTIFICIAL PRODUCTION OF SPORES IN MONAS**





## THE ARTIFICIAL PRODUCTION OF SPORES IN MONAS BY A REDUCTION OF THE TEMPERATURE

ARTHUR WHITE GREELEY

THE processes of reproduction among the Protozoa present many interesting problems from the physiological point of view. Aside from the so-called sexual reproduction, which is always preceded by the conjugation of two similar or slightly differentiated individuals, there exist many forms of asexual reproduction, varying from the simple division of the parent organism into two, or in some cases as many as eight, daughter cells, to the more complicated processes of encystment and spore formation. The processes of encystment and spore formation may be preceded by conjugation, but in most cases they are simply a direct transformation of the motile organism into a resting form. Surprisingly little is known about the physical and chemical conditions which determine the transformation of Protozoa into cysts and spores.

It has generally been observed that when a pond in which the organisms live begins to dry out, cysts are formed. This fact caused Cienkowski<sup>1</sup> to undertake a series of experiments. He kept cultures of Infusoria in small, loosely covered dishes, and allowed the water gradually to evaporate. Before the evaporation had become complete, all the Infusoria had formed cysts. Other investigators attributed this encystment to a lack of oxygen, rather than to the evaporation of the water. Maupas<sup>2</sup> has kept cultures of various Infusoria for long periods of time, and has found that some of the carnivorous forms, notably Oxytrichia, form cysts after they have been deprived of food. Hertwig<sup>3</sup> has observed that the same fact holds good for Actinosphærium. In the same series of experiments he found, however, that the consumption of an excess of food may cause encystment as well as starvation. Other cases are on record also in which various carnivorous Infusoria have been seen to encyst after engulfing a large amount of food. Klebs,<sup>4</sup> in a large number of experiments on fresh-water Algæ, has observed that in Vaucheria zoöspores are formed when the filaments are transferred from the light to the dark. Klebs reared Vaucheria in the following solution, used at concentrations of from 0.1 to 0.4 per cent.:  $\text{Ca}(\text{NO}_3)_2$ , four parts;  $\text{MgSO}_4$ , one part;  $\text{KNO}_3$ , one part;  $\text{K}_2\text{HPO}_4$ , one part; and found that when the filaments were transferred from this solution to distilled water, irrespective of light or dark, zoöspores were formed. He also succeeded in producing parthenogenetic spores in Spirogyra by plasmolyzing the cells with a sugar solution. Klebs makes the general statement that the formation of zoöspores in Vaucheria is aided by lowering the temperature,

<sup>1</sup> CIENKOWSKY, *Archiv für mikroskopische Anatomie*, Vol. I (1866), p. 203.

<sup>2</sup> MAUPAS, *Archives de zoologie expérimentale*, Vol. VI (1888), p. 165.

<sup>3</sup> HERTWIG, *Sitzungsberichte der Gesellschaft für Morphologie und Physiologie in München*, 1899.

<sup>4</sup> KLEBS, *Die Bedingungen der Fortpflanzung bei einigen Algen und Pilzen*, Jena, 1896.

but made no experiments to show that a lowering of the temperature itself will actually cause the plant to form spores. He apparently had in mind only the limits of temperature at which spore formation may take place, when the process is initiated by other means. Beyond these observations, which are rather inconclusive so far as the Protozoa are concerned, nothing is known about the conditions which determine encystment or spore formation.

Dr. Loeb suggested that I take up the problem of asexual reproduction from an experimental point of view. Many authors had noticed that the mode of reproduction changes in certain aquatic animals or Protozoa when the pond in which they live begins to dry out. But the question was: How can the lack of water in a pond interfere with the mode of reproduction? Dr. Loeb's idea was that the real physical factor at work in this case was the rapid, or extensive, changes of temperature. As long as



FIG. 1. THE ADULT MONAS

the bulk of water in a pond is large, the daily changes of the temperature of the air will cause only a slow or slight variation in the temperature of the pond. But when the bulk of water is small, the temperature of the latter will follow sudden changes in the temperature of the air more rapidly and completely. In order to test this idea, he suggested that I try whether or not, through sudden changes of temperature, organisms might be caused at any time to reproduce asexually instead of sexually. The experiments were first performed on *Stentor*, with the results already described in a previous paper.<sup>5</sup> It was found in these experiments that by lowering the temperature the animal would go into a resting stage, which in appearance resembled a cyst; but in no case did I obtain spores. These results could not be obtained by raising the temperature.

During the past year the low-temperature experiments have been continued on several other Protozoa, and in all of them structural changes similar to those already described for *Stentor* have been obtained. But in one form, *Monas*, our original purpose has been carried out, namely, the artificial production of spores by means of variations in the temperature.

*Monas* is a small flagellated Infusorian, of an exceedingly simple structure, and occasionally appears in great numbers in cultures that have been prepared for *Paramecia*. It can be easily maintained in the laboratory in great quantities by adding to the culture from time to time a little bread, upon which the *Monads* thrive surprisingly. In all the experiments the *Monads* were isolated in small, covered dishes, and the supply of water kept constant by frequent renewal from the aquaria in which the animals had been reared. The temperature was lowered to the desired point by placing

<sup>5</sup> GREELEY, *American Journal of Physiology*, Vol. VI (1901), p. 122.

the dishes in a refrigerator in which constant temperatures, ranging from  $1^{\circ}$  to  $10^{\circ}$  C., could be maintained. For each low-temperature experiment a control experiment was performed at the temperature of the room, and great care was taken that all the conditions, with the exception of temperature, should be identical in the two cases.

Monas is more sensitive to changes in the temperature than any of the other Protozoa experimented with. Within a few hours after the temperature has been

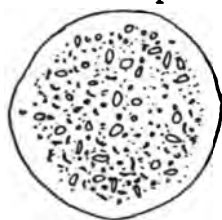


FIG. 2

A resting cell of Monas, formed by an exposure to a temperature of  $4^{\circ}$  C. during six hours.

lowered to  $4^{\circ}$  C. all the Monads in a dish settle to the bottom and cease their progressive movement. At the same time the cell gradually becomes spherical, the flagellum and mouth-opening disappear, and there is formed a resting cell like those already described in the experiments upon Stentor. These resting cells can be kept at a temperature of  $4^{\circ}$  to  $6^{\circ}$  C. indefinitely, and will withstand partial desiccation without losing their power to revert to the normal Monas form when they are removed to the temperature of the room. This reversion to the motile form takes place within twenty-four hours after the room temperature has been reached. The flagellum first makes its appearance and the cells become motile while still in the spherical condition. They soon, however, assume the normal elongated form of the adult Monas.

If these resting cells of Monas which have been formed at a temperature of  $4^{\circ}$  to  $6^{\circ}$  C., instead of being returned to the temperature of the room, be placed on ice at a temperature of  $1^{\circ}$  C., further structural changes take place as a result of this extreme lowering of the temperature. After remaining at a temperature of  $1^{\circ}$  C. for five to seven days, the protoplasmic contents of the resting cells break up into small spherical spores, from two or three to twenty-five in each cell. In most cases these spores are discharged from the resting cells as soon as they are formed. They have thick cell walls, and are non-motile. They may be kept indefinitely at any temperature below  $8^{\circ}$  C., and withstand desiccation perfectly.

When the spores are removed to the temperature of the room and isolated in small, closed cells under the microscope, their development into the motile Monad can be easily followed. The first attempts to demonstrate the development of the spores failed in several instances because of a lack of oxygen in the closed cells in which the spores were isolated, due to the presence of motile Monads which originated from resting cells isolated with the spores. But finally, at Dr. Loeb's suggestion, some fresh-water Algæ were mixed with the spores as soon as they were returned to the temperature of the room. In this way a supply of oxygen was maintained, and the development of the spores began at once. The first change that can be observed is the appearance of a thin layer of protoplasm which grows out of the spore. This protoplasmic layer develops into a small, spherical cell, which gradually becomes separated from the spores. The cell is at first hardly visible,

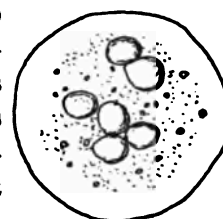


FIG. 3

The formation of spores within the resting cell after an exposure to a temperature of  $1^{\circ}$  C. for five days.

because of the extreme transparency of its protoplasm, but it eventually becomes granular and develops a flagellum, which appears to originate as a long, delicate pseudopod, extending outward from the protoplasm. The Monad formed in this way finally becomes motile and swims away, leaving behind the empty spore or capsule. In some cases the spores are not discharged from the resting cell as soon as they

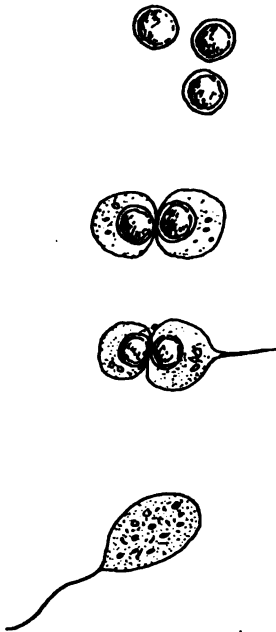


FIG. 4  
Four stages in the development of the spore into the motile Monad.

are formed, but develop within its walls, so that it is possible to find spores in all stages of development, even the young Monads, within the old resting cell. The development of the spores usually occupies from two to four days, although in some cases it may be delayed for a much longer time. The Monads formed from the spores are at first spherical, and swim about with the characteristic revolving movement of this species of Monas. The cells rapidly become elongated, however, and within a short time are indistinguishable in all respects from the adult organism.

A few typical experiments will be briefly described, as follows:

*Experiment I.*—On April 11 a culture of Monas was placed in the refrigerator at a temperature of 1° to 4° C. The next day examination showed that all the Monads had formed spherical resting cells. Some of these were removed to the temperature of the room, and they immediately developed into the motile form. On April 24 many of the resting cells had broken up into spores. A small number of these resting cells and spores were isolated under the microscope. The resting cells immediately developed into the motile Monads, as before. On April 26 a few of the spores had formed small Monads, but the development did not go very far because of a lack of oxygen in the closed cells which contained the spores.

*Experiment II.*—On April 18 a culture of Monas was put on ice at a temperature of 10° C. On May 3 the culture was removed to a temperature of 6° C. A large number of spores had been formed, a large proportion of them remaining within the resting cells. On May 6 many of the spores had developed into young Monads. A large number of the Monads were found swimming about within the walls of the resting cells.

*Experiment III.*—On May 14 a culture of Monas was placed on ice at a temperature of 1° C. On May 22 a large number of the resting cells and spores were removed to the room temperature and isolated under the microscope in closed cells that contained fresh-water Algae. Development of the spores commenced at once, and on May 25 they had reached the motile Monad form.



FIG. 5  
Young Monads developed from the spores within the old cell wall of the resting cell.

## SUMMARY

1. In *Monas* a reduction of the temperature brings about certain structural changes within the cell that result in the formation of many small spores, each of which has the power of reproducing the organism.

2. It is thus possible by means of variation in the temperature to control the methods of reproduction in *Monas*. At a temperature of 20° C., *Monas* multiplies sexually and by simple fission. At a temperature of 1° to 4° C., reproduction by asexual spores takes place.



## **THE SELF-PURIFICATION OF STREAMS**





## THE SELF-PURIFICATION OF STREAMS

EDWIN O. JORDAN

It has long been a popular belief that "running water purifies itself," and that a polluted stream again becomes pure after flowing for some distance from the point of defilement. This widespread belief appears to be based on the evidence of the senses and to depend largely upon naked-eye inspection of the flowing stream. The most casual observer can often detect evidences of recent pollution at or immediately below the point where fouling of the water occurs, but farther down stream these indications of contamination become less striking, and farther still every trace of them vanishes.

Such observations are, however, open to misinterpretation. The mixing of the befouled water with the purer water of the river becomes more complete the greater the distance from the source of pollution, and when the increase in the volume of the stream from tributaries, and especially from underground sources, is also taken into consideration, a sufficient explanation is afforded for the changes observed. The thorough mingling of the polluting matters with the total volume of water in the stream, combined with a continuous and often rapid increase in the volume of the stream itself, may dilute sewage to such a degree that it seems as if the polluting substances had been materially lessened or had altogether disappeared. It is quite evident, however, that, while such dilution may improve the general appearance of a river water, it does not by any means follow that noxious elements introduced with the pollution are destroyed. Typhoid bacilli, for example, that enter a sewage-polluted river may persist in the river water, so far as the unaided senses can determine, for many miles below the point of entrance, although the water of the stream may to all outward appearances have quickly regained its pristine purity. Dilution in itself can neither remove nor destroy dangerous substances; it may, in fact, mask their presence.

A necessity arises, therefore, for a more searching study of the conditions existing in a sewage-polluted river. One method of investigation that has often been applied to this study consists in a determination of the chemical changes that occur in the flowing water. The amount of organic matter in water, especially the organic nitrogenous matter, can be determined by analytical processes of great delicacy and precision. It is found that fresh sewage or sewage-polluted water contains a relatively high proportion of organic nitrogenous matter in an unstable condition. "Organic nitrogen," or "albuminoid ammonia," and "free ammonia" are present in great abundance in the water of streams freshly contaminated with sewage. The putrescible nature of these substances, due to their liability to offensive bacterial decomposition, imparts to polluted water many of its characteristic qualities. Analyses have shown that a diminution in the amount of these organic substances occurs during the pas-

sage of the water down stream, and that hand in hand with the lessening of "organic nitrogen" and "free ammonia" in the water there is to be noticed first an increase in "nitrites" and then in "nitrates," until eventually a large part of the unstable organic nitrogen entering the river in sewage becomes oxidized into the stable mineral condition of nitrate. When the nitrogen has reached the state of nitrate—that is to say, when nitrification is complete—the river water may be said to be chemically purified, and, unless fresh organic matter is introduced or is formed by algal growth, is no longer capable of giving rise to offensive decomposition products.

This process of chemical purification is an important one, and is most influential in restoring a polluted stream to its original condition. Since the oxidation of the free ammonia and nitrites, as well as of the organic nitrogen, is due to bacterial activity, there is a falling off in the number of bacteria as their food-supply lessens; and thus it happens that in a general way the lines of chemical and bacterial purification follow a parallel course. In a freshly polluted river the number of bacteria found in one cubic centimeter of the water may be as high as several millions, while in the same river, after oxidation has taken place and the nitrogen has passed into the fully oxidized condition, the bacteria may number only a few thousand. It need hardly be pointed out perhaps that dilution tends to reduce the proportion both of organic matter and of bacteria in a given quantity of water. The evidence is, however, entirely convincing that, in addition to this relative diminution, there is also an absolute loss in the quantity of organic nitrogen and an absolute destruction of bacterial life in the flowing stream.

It may properly be asked: What is the precise sanitary significance of these changes that have just been described? Are the rate of oxidation of nitrogenous organic matter and the death-rate among river bacteria to be taken as legitimate criteria of an increasing wholesomeness of the water? The question is difficult to answer. Under ordinary conditions in this country the chief water-borne disease is typhoid fever. Stripped of all technicalities, the real question at issue on the sanitary side in the self-purification of streams is this: How far can typhoid bacilli travel in a flowing stream?

It is probably true that the increasing freedom of river water from typhoid germs as the point of pollution is left behind corresponds roughly with the increase in nitrates and the diminution of free ammonia and nitrites in the water; but there is no necessary connection. The observation that there is often coincidence between the state of the nitrogenous constituents in a water and the wholesomeness of that water is due to the fact that in a recently polluted water not only free ammonia and nitrites are likely to be present, but also typhoid bacilli. In a water in which pollution is more remote both free ammonia and typhoid bacilli are more likely to have disappeared.

The actual number of bacteria of all kinds in a river water is possibly a more warrantable standard of the degree of purification that has occurred than the state of the chemical substances in such a water. There is no escape from the conclusion that the duration of life of the ordinary sewage bacteria, when these are introduced into

water, measures the probable duration of life of the typhoid bacillus with greater accuracy than does the progressive oxidation of organic nitrogenous substances. At the same time it must be remembered that both methods are inferential only. There is no reason for believing that either such amounts of "organic nitrogen" or of "nitrites," or such numbers of bacteria as are ordinarily found in a polluted river, are in themselves directly harmful. Their presence and abundance simply furnish indications, more or less cogent, for gauging the probability of occurrence of typhoid bacilli.

In connection with the writer's study of the conditions attending the opening of the Chicago Drainage Canal, it became necessary to review the evidence for the self-purification of streams and to consider the practicability of applying to the study of the problem other methods than those just cited. The direct and ideally preferable method would consist in determining the proportion of typhoid bacilli in sewage, and then tracing the fate of these bacilli in the water course; but this is unfortunately not applicable, owing to the practically insurmountable difficulty of rapidly isolating and identifying the typhoid bacillus in the presence of large numbers of sewage and water bacteria. Several existing methods for the isolation of the typhoid bacillus from water were tested and proved entirely inadequate for this purpose.

Another method was accordingly employed, which has yielded results of interest and value. This consists in a determination of the relative abundance of *B. coli communis* in the river water at various points. *B. coli*, as is well known, occurs in large numbers in fresh sewage, and its presence can easily be detected by appropriate tests. The peculiar importance attaching to the fate of this microbe in a flowing stream lies in its close biological relationship to the typhoid bacillus, and in the fact that, like the typhoid bacillus, it enters river water with sewage. It is, moreover, invariably present in sewage in much larger numbers than the typhoid bacillus. Nearly all of the colon bacilli and probably all of the typhoid bacilli found in sewage pass directly into the sewage from the human body. Conditions, therefore, that affect the abundance of the colon bacillus in water are likely to affect that of the typhoid bacillus also.

It is not necessary to rehearse the general circumstances of the investigation, since these have been set forth with sufficient detail elsewhere.<sup>1</sup> References to the accompanying figure (Fig. 1) will show the principal points of collection of water samples. The methods employed for the detection of the colon bacillus must, however, be briefly considered. The necessity for handling a large number of water samples in a limited time led to the use of some method which could be applied with a fair degree of exactness to routine work. Both the carbol-broth method<sup>2</sup> and the fermentation-tube method<sup>3</sup> have been used. In much of the earlier work the cultures isolated by these two methods were worked out in detail, so that complete identification

<sup>1</sup> *Journal of Experimental Medicine*, Vol. V (1900), p. 271.

<sup>2</sup> *Journal of Hygiene*, Vol. I (1901), pp. 295-320.

<sup>3</sup> SMITH AND BROWN, "Report on Mohawk and Hudson Rivers," *Thirtieth Annual Report of the State Board of Health of New York*, 1893, p. 680.

of the bacterial species was secured. The exigencies of the later work brought about the partial abandonment of this procedure and compelled the adoption of a rough field method by means of which large numbers of water samples could be treated with a limited margin of error. The dextrose fermentation tube proved the most available for this purpose, experiments with neutral-red<sup>4</sup> and other methods not yielding, on the

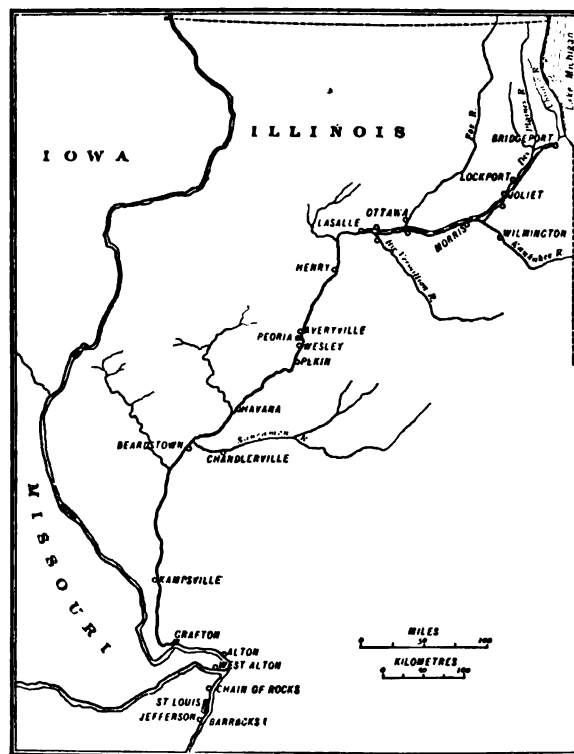


FIG. 1

whole, as satisfactory results. The fermentation tube was used in this way to some extent in routine work in 1899–1900, and was employed as the sole method in a more extended series of tests in the autumn of 1901. The interpretation adopted for the changes produced by inoculating water into the fermentation tube was as follows: Positive reactions—*i. e.*, those indicating the presence of *B. coli*—were regarded as those tubes showing gas production amounting to over 20 per cent. of the tube length, the tubes yielding on absorption with NaOH a gaseous residue (H) appreciably in excess of the  $\text{CO}_2$  absorbed; negative reactions were those showing (a) no gas production, or (b) gas production less than 10 per cent. of the tube length; the doubtful class was made to include (a) those tubes yielding only 10–20 per cent. of gas, and (b) those yielding more than 20 per cent., but with an appreciable excess of  $\text{CO}_2$ .

<sup>4</sup> E. E. IRONS, *Journal of Hygiene*, Vol. II (1902), p. 314.

Any method susceptible of rapid application, like the one outlined, leads to occasional misinterpretation, but the following data indicate that where a large number of water samples are treated the error is not unduly large. The organisms were isolated from a series of fermentation tubes and studied in detail, and complete identification was arrived at. The procedure is illustrated in Table I:

TABLE I

Date collected	Date examined	Source <sup>5</sup>	Sample number	Amount of water	Gas PRODUCTION <sup>6</sup>		Absorption H-CO <sub>2</sub>	Culture. Number of org. iso.	Interpretation	Organisms isolated
					24 hours	48 hours				
Oct. 23	Oct. 23	Three	1039	1 c.c.	10	30	2-1	1039 x	+	+ B. coli
25	25	Four	1044	1-100 c.c.	16	32	1-1	1044 y	?	- B. cloacæ
28	28	Eight	86	1 c.c.	25	25	4-1	86 y	+	- B. proteus
30	30	Two	2032	1-1000 c.c.	38	43	2-1	2032 x	+	+ B. coli
Nov. 4	Nov. 4	One	2039	1-10 c.c.	36	62	1-2	2039 x	?	- B. cloacæ
4	4	Four	1060	1-10 c.c.	20	85	2-1	1060 x	+	+ B. coli
8	8	Two	2048	1-1000 c.c.	42	67	3-1	2048 z	+	+ B. coli
19	19	Six	180	1 c.c.	5	95	1-1	180 z	?	+ B. coli
19	19	Seven	179	1 c.c.	20	45	1-1	179 z	?	- B. cloacæ
Oct. 11	Oct. 11	Three	1019	1-10 c.c.	5	8	-	1019 x	-	- B. proteus
Nov. 20	Nov. 20	Two	2065	1-1000 c.c.	20	35	3-1	2065 x	+	+ B. coli
22	22	One	2071	1 c.c.	5	15	No absp.	2071 x	?	- B. proteus
16	16	Four	1082	1-10 c.c.	15	25	4-1	1082 z	+	+ B. coli
Dec. 5	Dec. 5	One	2091	1-10 c.c.	82	82	2-1	2091 x	+	+ B. coli
16	16	One	2109	1 c.c.	90	94	2-1	3109 x	+	+ B. coli
27	27	One	2127	1-10 c.c.	5	22	2-1	{ 2127 y 2127 y	+	{ + B. coli B. proteus

Sixty-three tubes were examined in this way, with the following results:

- 39 Interpretation positive. Typical B. coli isolated.
- 7 Interpretation positive. B. coli not isolated.
- 9 Interpretation doubtful. B. coli not isolated.
- 4 Interpretation doubtful. Typical B. coli isolated.
- 4<sup>7</sup> Interpretation negative. B. coli not isolated.

The satisfactory application of this method to the problem of the self-purification of streams depends upon ascertaining in each case the dilution at which the test gives uniformly positive and that at which it gives uniformly negative results. For illustration, sixty-nine separate examinations of .001 of a cubic centimeter of the Mississippi river water at Grafton were made in October-December, 1901, and in no case was the presence of B. coli detected in this quantity of water. Unless the negative limit is ascertained on each day, it is apparent that the results lose almost all their significance. The mere fact that B. coli is "present" in 1 c.c. of water is without meaning

<sup>5</sup> See Fig. 2, p. 11.

<sup>6</sup> Percentage of tube length.

<sup>7</sup> These were all cases showing less than 10 per cent. of

gas in which gas-forming organisms — not B. coli — were isolated. In addition, some twenty tubes were examined which showed growth in the closed arm, but yielded no gas-producing organism of any kind.

unless at the same time it is shown to be absent in a small amount (.1 c.c.) of the same water. On the other hand, any attempt to reduce the findings to an exact numerical basis, even if it were possible, would be of doubtful value. No sanitary importance can be attached to slight variations in the colon content, such a difference, for instance, as between thirty and forty per cubic centimeter being probably devoid of significance; but the greater diversity revealed by the decimal dilution is unquestionably fraught with meaning. The method here used has the advantage of recording the more con-

TABLE II  
ILLINOIS RIVER (AVERYVILLE)  
Presence of Bacteria of the Colon Group

DATE	SERIAL No.	.001 c.c.	.01 c.c.	.1 c.c.	1 c.c.	DATE	SERIAL No.	.001 c.c.	.01 c.c.	.1 c.c.	1 c.c.
		+ - ?	+ - ?	+ - ?	+ - ?			+ - ?	+ - ?	+ - ?	+ - ?
Oct. 19	2013	1	2	1	.....	Nov. 27	2079	.....	.....	1	3
21	2015	1	2	1 1	.....	29	2081	.....	.....	2	2
22	2017	1	1 1	1 1	.....	30	2083	.....	1	1	1
23	2019	.....	3	1	1	Dec. 2	2085	.....	.....	1 1	1
24	2021	.....	3	2	.....	3	2087	.....	1	2	1
25	2023	.....	3	2	.....	4	2089	.....	1	1 1	1
26	2025	.....	2	2	.....	5	2091	.....	1	2	1
28	2027	.....	3	1	1	6	2093	.....	1	2	1
29	2029	.....	1		2	7	2095	.....	1	2	1
30	2031	.....	2	2	.....	9	2097	.....	1	1	1
31	2033	.....	2	2	.....	10	2099	.....	1	2	1
Nov. 1	2035	.....	1 1	2	.....	11	2101	.....	1	1 1	1
2	2037	.....	2	2	.....	12	2103	.....	1	1 1	1
4	2039	.....	1 1	1	1	13	2105	.....	1	1	1
5	2041	.....	2	1	1	14	2107	.....	1	2	1
6	2043	.....	2	1	1	16	2109	.....	1	1 1	1
7	2045	.....	2	1	1	17	2111	.....	1	1 1	1
8	2047	.....	2	2	.....	18	2113	.....	1	1 1	1
9	2049	.....	2		1 1	19	2115	.....	1	2	1
11	2051	.....	1 1	1 1	.....	20	2117	.....	1	2	1
12	2053	.....	1	1 1	1	21	2119	.....	1	1	1
13	2055	.....	1	1 1	1	23	2121	.....	1	1 1	1
14	2057	.....	1	1 1	1	24	2123	.....	1	1 1	1
15	2059	.....	1	1 1	1	26	2125	.....	1	2	1
16	2061	.....	1	1 1	1	27	2127	.....	1	2	1
18	2063	.....	.....	2	1	28	2129	.....	1	1 1	1
19	2065	.....	.....	2	2	30	2131	.....	1	1 1	1
20	2067	.....	.....	1	2						
21	2069	.....	.....	1	2 1						
22	2071	.....	.....	1	1 1 1						
23	2073	.....	.....	2	2						
25	2075	.....	.....	2	1 1						
26	2077	.....	.....	1	1 1						
								No. days examinat's	3	49	60
								No. days B. coli f'nd	0	3	38
								No. determinations	3	72	114
								No. positive results	0	4	49
								Per cent. pos. results	0%	6%	43%

siderable differences and fluctuations in the colon content without obscuring the issue by a pretense to greater accuracy than can be obtained by any existing methods.

The mode of employment of the method may be shown by the record of fermentation-tube work upon the water of the Illinois river at Averyville (Table II).

Two series of determinations have been made in this way, one in 1899-1900, elsewhere described,<sup>8</sup> and the other in 1901. The results may be set forth most clearly in tabular form (Tables III-VII):

TABLE III<sup>9</sup>  
Principal Stations on the Illinois River, 1899-1901

COLLECTING STATION	.00001 c.c.		.0001 c.c.		.001 c.c.		.01 c.c.		.1 c.c.	
	No. of days water exam'd	No. of days B. coli found	No. of days water exam'd	No. of days B. coli found	No. of days water exam'd	No. of days B. coli found	No. of days water exam'd	No. of days B. coli found	No. of days water exam'd	No. of days B. coli found
Ill. and Mich. Canal, Lockport.....	28	7	32	28	11	8	4	4	2	2
Illinois river, Morris .....	..	..	3	1	20	11	30	20	23	20
Illinois river, Ottawa.....	..	..	..	..	..	..	22	6	34	19
Illinois river, Averyville.....	..	..	..	..	..	..	1	0	27	4
Illinois river, Wesley City.....	..	..	..	..	7	1	22	3	28	13
Illinois river, Grafton.....	..	..	..	..	..	..	4	1	35	13

TABLE IV<sup>9</sup>  
Illinois River at Averyville and Grafton Compared with Tributaries and with the Mississippi (Grafton) and Missouri (West Alton) Rivers, 1899-1900

COLLECTING STATION	.01 c.c.		.1 c.c.		1 c.c.		5 c.c.	
	No. of days water exam'd	No. of days B. coli found	No. of days water exam'd	No. of days B. coli found	No. of days water exam'd	No. of days B. coli found	No. of days water exam'd	No. of days B. coli found
Illinois river, Averyville.....	1	0	27	4	31	13	..	..
Illinois river, Grafton.....	4	1	35	13	38	26	4	2
Mississippi river, Grafton.....	2	0	34	10	35	23	4	3
Desplaines river.....	..	..	8	1	5	2	..	..
Kankakee river.....	..	..	6	3	5	4	..	..
Fox river.....	..	..	22	2	23	6	13	10
Big Vermilion river.....	..	..	5	1	9	3	..	..
Sangamon river.....	13	4	25	14	27	21	..	..
Missouri river.....	6	3	32	13	31	21	..	..

<sup>8</sup>See footnote 2, p. 5.

<sup>9</sup>It will, of course, be observed that this method of summarizing the results is not altogether precise. The fact that on certain days and with certain dilutions more than one determination was made obviously implies the examination of a larger quantity of water at those times and the

increased possibility of a positive finding. The tabulation of the results on the basis of the total number of determinations is, however, open to objection on other grounds, and the method I have employed seemed to me on the whole to present fewer disadvantages.



TABLE V<sup>10</sup>  
1899-1900

	.1 c.c.		1 c.c.	
	No. of days water exam'd	No. of days B. coli found	No. of days water exam'd	No. of days B. coli found
Total, Illinois river (Averyville and Grafton).....	62	17	69	39
Total, tributaries of Illinois river.....	66	21	69	36
Total, Mississippi and Missouri rivers .....	66	23	66	44

TABLE VI  
October-December, 1901

COLLECTING STATION	.0001 c.c.		.001 c.c.		.01 c.c.		.1 c.c.		1 c.c.		2 c.c.		5 c.c.	
	No. days water examined	No. days B. coli found	No. days water examined	No. days B. coli found	No. days water examined	No. days B. coli found	No. days water examined	No. days B. coli found	No. days water examined	No. days B. coli found	No. days water examined	No. days B. coli found	No. days water examined	No. days B. coli found
Illinois river, Averyville .....	..	..	3	0	49	3	60	38	41	34	..	..	..	..
Illinois river, Pekin.....	17	4	44	14	44	40	17	17	..	..	..	..	..	..
Illinois river, Grafton.....	..	..	52	4	64	6	64	12	53	19	13	5	16	8
Mississippi river, Grafton....	..	..	52	0	64	6	62	25	54	25	12	4	16	11
Miss. r., Chain, Ill. shore.....	..	..	25	0	41	3	41	8	42	25	..	..	7	5
Miss. r., Chain, mid-stream...	..	..	27	0	41	4	41	16	42	35	..	..	7	6
Miss. r., Chain, intake tower..	..	..	27	0	43	11	42	30	43	36	6	6	5	5
Miss. r., Chain, Mo. shore....	..	..	27	1	41	8	41	24	41	36	..	..	4	4
Missouri r., Bellefontaine....	..	..	32	1	44	13	44	31	43	34	..	..	8	7

TABLE VII  
October-December, 1901

COLLECTING STATION	.0001 c.c.			.001 c.c.			.01 c.c.			.1 c.c.			1 c.c.			2 c.c.			5 c.c.		
	No. determinations made	No. positive results	Percentage positive results	No. determinations made	No. positive results	Percentage positive results	No. determinations made	No. positive results	Percentage positive results	No. determinations made	No. positive results	Percentage positive results	No. determinations made	No. positive results	Percentage positive results	No. determinations made	No. positive results	Percentage positive results	No. determinations made	No. positive results	Percentage positive results
Illinois river, Averyville..	..	..	..	3	0	0	72	4	6	114	49	43	54	39	72	..	..	..	..	..	..
Illinois river, Pekin.....	25	7	28	75	16	21	77	60	78	17	17	100	..	..	..	..	..	..	..	..	..
Illinois river, Grafton .....	..	..	..	72	5	7	117	6	5	125	16	13	71	21	30	15	5	33	17	8	47
Mississippi river, Grafton..	..	..	..	69	0	0	115	6	5	123	29	24	74	28	38	12	4	33	16	11	69
Miss. r., Chain, Ill. shore..	..	..	..	25	0	0	66	3	5	80	8	10	42	25	60	..	..	..	7	5	71
Miss. r., Chain, mid-stream	..	..	..	27	0	0	68	4	6	82	18	22	43	35	81	..	..	..	7	6	86
Miss. r., Chain, intake tower	..	..	..	27	0	0	71	14	20	84	48	57	43	36	84	6	6	100	5	5	100
Miss. r., Chain, Mo. shore..	..	..	..	27	1	4	68	9	13	81	33	41	42	37	88	..	..	..	4	4	100
Missouri r., Bellefontaine..	..	..	..	34	1	3	84	16	19	95	48	50	44	35	79	..	..	..	8	7	88

<sup>10</sup> See footnote 9, p. 9.

The situation of the collecting stations on the lower Illinois, Mississippi, and Missouri rivers is shown by the accompanying map.

If any weight is to be attached to the relative abundance of the colon bacillus in river water, it is clear from the data here presented that the water of the Illinois river undergoes a real and very considerable purification. The colon bacteria which are

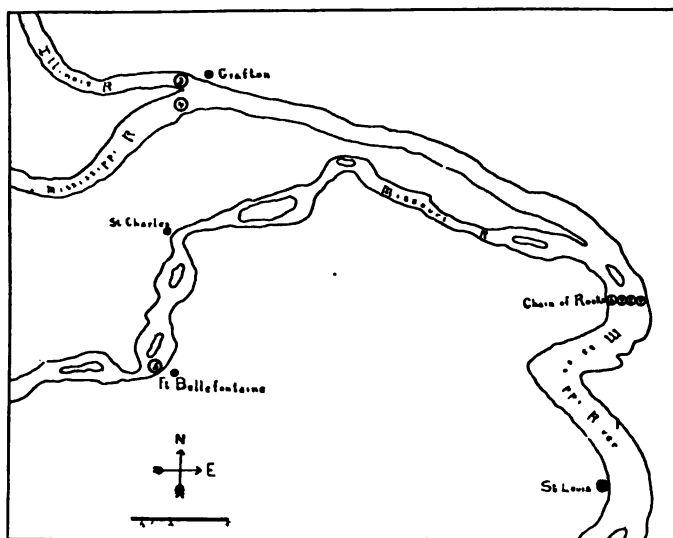


FIG. 2

present in such large numbers in Chicago sewage (*cf.* Table III) disappear almost completely in less than 150 miles' flow. At the mouth of the Illinois river, despite the enormous initial pollution and the very large secondary pollution at Peoria (Tables III, VI, and VII), the number of colon bacteria is certainly no greater than the number in the Mississippi river (*cf.* .01 c.c., Table VII), and perhaps not as large as the number in the Missouri river. Since all investigators are agreed that the colon bacillus is more hardy than its relative, the typhoid bacillus, and can live in water for a longer time, there is every reason for supposing that the latter microbe dies out with at least the same rapidity.

If it be true that the fate of the colon bacillus in running water furnishes the most satisfactory indication we can secure at present of the continuance of vitality of the typhoid bacillus, there can be no hesitation as to the conclusions to be drawn from our investigation. Since this near biological relative of the typhoid bacillus perishes speedily and in large numbers in the course of the Illinois river, there is reason to suppose that the typhoid bacillus itself does not long survive exposure to the same conditions.



## **THE LECITHANS**



## THE LECITHANS

### THEIR FUNCTION IN THE LIFE OF THE CELL

WALDEMAR KOCH

THE ash left on the incineration of tissues obtained from various parts of the body, especially the brain, has long been known to contain phosphorus. Of the chemical combination in which this phosphorus was present in the original tissues nothing was known until Gobley<sup>1</sup> carefully studied an organic phosphorus-containing body, which he isolated from eggs and called lecithin. He obtained as splitting products glycerophosphoric acid and some of the fatty acids. Diaconow<sup>2</sup> continued this work at the suggestion of Hoppe Seyler, and isolated as splitting products glycerophosphoric acid, stearic and oleic acids, and a base which he identified with Baeyer's neurin and the neurin obtained by Liebreich<sup>3</sup> from his protagon by decomposition with barium hydrate. From the ease with which his lecithin could be split up Diaconow concluded that it was a neurin salt of distearyl glycerophosphoric acid. This view was, however, disproved by Hundeshagen<sup>4</sup> on account of the fact that the body prepared by the union of neurin and distearyl glycerophosphoric acid in alcohol solution would not give the characteristic myelin forms, although it possessed all the other properties of lecithin. Strecker<sup>5</sup> brought confusion into this subject by identifying the base obtained by him from lecithin with the cholin he had isolated from bile.<sup>6</sup> Thudichum<sup>7</sup> pointed out the difference between the body derived from bile and the base isolated from lecithin, and identified the latter as neurin by a number of analyses made with carefully purified material. In the book above referred to Thudichum also records some other important observations. Among the large number of compounds isolated by him from the brain there are some which do not contain glycerin; as he always finds phosphorus in the form of orthophosphoric acid, he concludes to call these bodies "Phosphatids" and consider them rather as derivatives of orthophosphoric acid than glycerophosphoric acid, as previously accepted. His formulæ resemble the types of the *Typentheorie* of Gerhardt and Wurtz, in leaving the exact building up of the molecule a matter of doubt. From a study of the fatty acids in his various compounds Thudichum concludes, contrary to Diaconow, that there are no phosphatids with only one fatty acid, but that each one contains either palmitic, stearic, or mar-

<sup>1</sup> GOBLEY, *Journal de pharmacie et de chimie* (1850), Vol. XVII, p. 401, and Vol. XVIII, p. 107.

<sup>2</sup> DIACONOW, *Hoppe Seylers medicinisch-chemische Untersuchungen*, 1866, Vol. II, p. 221; also *Centralblatt für die medicinischen Wissenschaften* (1868), Vol. VI, pp. 2, 97, and 434.

<sup>3</sup> LIEBREICH, *Liebig's Annalen der Chemie und Pharmacie* (1865), Vol. CXXXIV, p. 34.

<sup>4</sup> HUNDESHAGEN, *Journal für praktische Chemie* (1883), Vol. XXVIII, p. 219.

<sup>5</sup> STRECKER, *Liebig's Annalen der Chemie und Pharmacie* (1868), CXLVIII, p. 78.

<sup>6</sup> *Ibid.* (1862), Vol. CXXIII, p. 353.

<sup>7</sup> THUDICHUM, *Die chemische Konstitution des Gehirns des Menschen und der Tiere*, 1901, p. 121.

gearic as one of the constituents; which, however, gives no character to the molecule, while the other acid—oleic for brain lecithin, kephalinic for kephalin—gives to the molecule its distinctive properties.

Without considering further the important question of the structure of these compounds, I would propose to classify them under the general term "Lecithans." The introduction of the word "lecithan" as a group name seems preferable to the use of an entirely new and unfamiliar term like "phosphatids," as proposed by Thudichum. At the same time, the change of the last syllable of lecithin to *an* gives sufficient variation to prevent any such confusion as attended the generalization of the word "albumen." The lecithans, then, are substances containing in the molecule phosphoric acid, fatty acids, nitrogen, and, in most cases, glycerin. They resemble each other very closely in their physical appearance, being waxy, non-crystalline, and very hygroscopic. Toward water they all show the same behavior, although their solubility or the solubility of their salts in *organic* solvents may vary.

The very general distribution of the lecithans in all forms of living tissues speaks for their value in the life of the cell. A more careful study of these compounds indicates that they are valuable in two ways: first, on account of their physical properties; and, secondly, on account of their chemical behavior.

#### PHYSICAL PROPERTIES

The behavior of the lecithans with water seems of especial interest, and can be watched under the microscope. A waxy piece of brain lecithin placed in water first swells up and then gives off long filaments (called myelins) which sometimes resemble a shepherd's crook, at other times a mass of twisted skein. If allowed to stand for some time, with frequent shaking, a perfect emulsion is finally formed. A lecithan which has been part of the living tissues, such as brain lecithin, gives a much more perfect emulsion than egg lecithin, which is merely stored food material. If such an emulsion is the substratum of the living cell—and there seems good reason to consider it so—it may explain some of the physical properties of living protoplasm. The study of this emulsion is especially interesting in connection with the changes in the physical conditions of the living cell brought about by electrolytes, as shown by the recent work of J. Loeb and his school.

*Action of electrolytes on an emulsion of brain lecithin.*—Four g. of brain lecithin (free from calcium, and containing less than 1 per cent. sodium or potassium) are treated with one liter distilled water. The resulting emulsion is sufficiently transparent for purposes of study, can be filtered unchanged, has a neutral reaction to litmus, and remains unaltered for weeks, especially after sterilization by boiling. The results of my experiments with this emulsion may be classified as follows:

*Univalent kations.*—Salts of Na, K,  $\text{NH}_4$ , Li, Ag, even in very concentrated solution, give no precipitate and have apparently no effect on the emulsion. The hydrogen ion is an exception, in the case of acids which are sufficiently dissociated. A

concentration of  $\frac{m}{200}$  sulphuric will give a precipitate. Carbonic acid is not sufficiently soluble to give any precipitate.

*Divalent kations.*—Mg, Ca, Sr, Ba, Co, Ni, Fe'', Zn, Cd, Cu, and Pb all give a precipitate which, similar to the one with acids, is flocculent, gelatinous, and settles to the bottom in less than one hour, leaving the supernatant liquid perfectly clear. The concentrations which will just give the precipitate vary somewhat and have been found in the case of Ca, Sr, and Ba to be  $\frac{m}{100}$ ,  $\frac{m}{40}$ , and  $\frac{m}{30}$ , respectively.

*Trivalent kations.*—Fe''', Al, Au give no precipitate and behave like monovalent kations. Cr gives unsatisfactory results. Au, after standing for several hours, is precipitated in the metallic state.

*Anions.*—Cl, Br, I, SO<sub>4</sub>'', oxalate, citrate, and ferrocyanide (K<sub>4</sub>Fe(CN)<sub>6</sub>) give no precipitate, and even in concentrated solution have no apparent effect on the emulsion. OH is an exception, causing the emulsion to clear up.

*Non-electrolytes.*—Albumins, peptones, glucose, urea, alkaloids, and narcotics like urethan and chloral give no precipitation reactions and leave the emulsion apparently unchanged. Chloroform has a tendency to be emulsified by the emulsion, a reaction which Thudichum had already observed with ether.

The precipitations above observed with the hydrogen ion and divalent kations seem to be of an entirely physical nature because:

1. They are independent of the concentration of the lecithin. An  $\frac{m}{2000}$  lecithin emulsion will begin to precipitate with about the same concentration of the divalent kation as an  $\frac{m}{200}$  emulsion. Stronger emulsions are not sufficiently transparent for observation.

2. Removal of the supernatant liquid by decantation and the addition of water will cause the precipitates to redissolve.

Cadmium, copper, and other salts of various lecithans have been prepared in alcohol solution and analyzed, but they are readily broken up on the addition of water, and belong to a class of physical compounds even more unstable than ordinary double salts. It would seem, then, that when a certain limiting concentration of the divalent kation is reached, the emulsion can no longer exist and the lecithin is precipitated, carrying with it possibly some of the salt. Very interesting, on account of the possibility of furnishing an explanation of such results as Loeb<sup>1</sup> obtains with *Fundulus*, are the antagonistic effects of univalent kations in preventing the precipitation. Near the limits at which Ca will just give a precipitate, a very small amount of Na will suffice to prevent this precipitate; as more Ca is added, relatively more Na is needed. A direct comparison of my results with J. Loeb's is not possible, because, in the first place, the amounts of Ca, Na, and lecithin in the *Fundulus* egg are not known, and, in the second place, the reaction between the solution and the egg does not come about as

<sup>1</sup> LOEB, *American Journal of Physiology* (1902), Vol. VI, p. 411.



directly as in my case. The following table gives the data obtained with an emulsion of brain lecithin:

				After Three Hours
I.	5 c.c. $\frac{m}{200}$ emul. + 5 c.c. water	+ 5 c.c. $\frac{m}{10}$ $\text{Ca}(\text{NO}_3)_2$	- - Immediate ppt.	Ppt. settled
II.	5 c.c. $\frac{m}{200}$ emul. + 5 c.c. 5 $m$ NaCl	+ 5 c.c. $\frac{m}{10}$ $\text{Ca}(\text{NO}_3)_2$	- - No ppt.	No ppt.
III.	5 c.c. $\frac{m}{200}$ emul. + 5 c.c. $\frac{m}{10}$ NaCl	+ 1.5 c.c. $\frac{m}{10}$ $\text{Ca}(\text{NO}_3)_2$	- - No ppt.	No ppt.
IV.	5 c.c. $\frac{m}{200}$ emul. + 5 c.c. $\frac{m}{10}$ NaCl	+ 5 c.c. $\frac{m}{10}$ $\text{Ca}(\text{NO}_3)_2$	- - Immediate ppt.	Ppt. settled
V.	5 c.c. $\frac{m}{200}$ emul. + 5 c.c. $\frac{m}{10}$ NaCl	+ 3.5 c.c. $\frac{m}{10}$ $\text{Sr}(\text{NO}_3)_2$	- - No ppt.	No ppt.
VI.	5 c.c. $\frac{m}{200}$ emul. + 5 c.c. 2 $\frac{1}{2}$ $m$ KCl	+ 5 c.c. $\frac{m}{10}$ $\text{Ca}(\text{NO}_3)_2$	- - No ppt.	No ppt.
VII.	5 c.c. $\frac{m}{200}$ emul. + 5 c.c. $\frac{m}{10}$ $\text{FeCl}_3$	+ 5 c.c. $\frac{m}{10}$ $\text{Ca}(\text{NO}_3)_2$	- - No ppt.	No ppt.
VIII.	5 c.c. $\frac{m}{200}$ emul. + 5 c.c. urea concentrated solution			
		+ 5 c.c. $\frac{m}{10}$ $\text{Ca}(\text{NO}_3)_2$	Ppt. formed slowly	Ppt. settled
IX.	5 c.c. $\frac{m}{200}$ emul. + 5 c.c. glucose concentrated solution			
		+ 5 c.c. $\frac{m}{10}$ $\text{Ca}(\text{NO}_3)_2$	Ppt. formed slowly	Ppt. settled

We may conclude, then, that the precipitation of lecithin by divalent kations is a physical phenomenon probably of an electrical nature, because:

1. Non-electrolytes do not prevent the precipitation (I, VIII, IX).
2. The trivalent kation  $\text{Fe}'''$  is much more efficient in preventing the precipitation than a monovalent one like Na (II, VII).
3. The precipitate is formed independent of the concentration of the lecithin and can be redissolved by the addition of water.

The application of these observations to Loeb's results must be postponed until other lecithans have been more carefully studied.

#### CHEMICAL PROPERTIES

The chemical properties of the lecithans depend on two groups in the molecule: first, the fatty acids, and, second, the complex of which the nitrogen is a part. The phosphoric acid, although the nucleus and very important in the building up of the molecule, does not seem to enter into any reaction, except on the complete destruction of the lecithan; as Halliburton<sup>9</sup> has found the phosphorus to decrease in degenerating nerves only after the eighth day.

Each lecithan contains, according to Thudichum, two fatty acids in the molecule: one—either palmitic, stearic, or margaric—does not impart any particular property to the compound; the other—oleic in the case of lecithin, kephalinic in the case of kephalin—gives to the molecule its distinctive character. This distinctive group is always unsaturated, will therefore add iodine, and bring about the reduction of osmic acid. Upon this group, then, depends the use of osmic acid as a stain for nervous

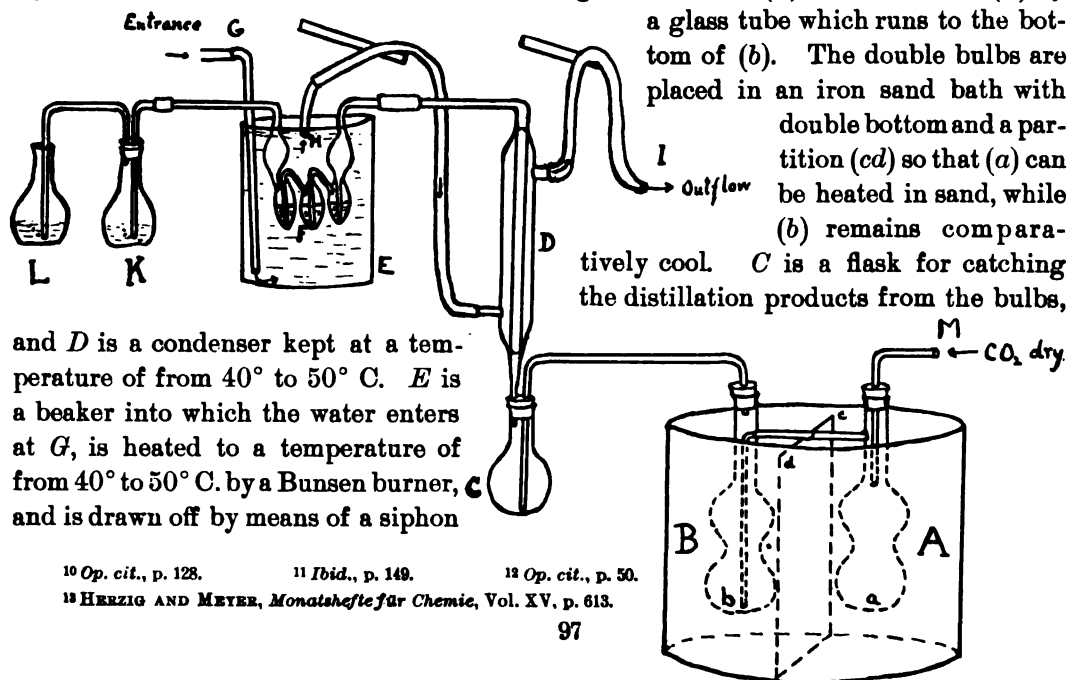
<sup>9</sup> W. D. HALLIBURTON, *The Chemical Side of Nervous Activity*, 1901, p. 87.

tissues in histological technique. The value of osmic acid as a general test for fats depends on the fact that all fats in the body contain some oleates. Pure stearates and palmitates will not give the test. The darkening of the lecithans on exposure to the air is also dependent on this group. In the case of kephalin, the change on exposure to the air takes place so rapidly as to suggest an autoxidizable substance capable of activating oxygen. The guiac-blue reaction, however, gives a negative result; and Thudichum<sup>10</sup> has shown that kephalin exposed to an atmosphere of oxygen in a eudiometer will not decrease the volume of the gas. The change is probably due to an internal rearrangement in the molecule, and takes place within the molecule of the fatty acid itself; as Thudichum<sup>11</sup> obtained from kephalin an acid by saponification (kephalinic acid) which exhibited the same changes as the mother-substance.

Less apparent, but nevertheless important, are the changes which the molecules of the lecithans undergo in the complex which contains the nitrogen. Thus Halliburton<sup>12</sup> has found the cholin to increase in the cerebro-spinal fluid as the result of general paralysis. For the quantitative investigation of the cholin or neurin the methyl groups attached to the nitrogen seem especially useful, as Herzig and Meyer<sup>13</sup> have devised a method by which such groups can be accurately determined. The description of the method is not easily accessible. I will therefore repeat it here, with such modifications as have been found useful, before going on to describe the results obtained with lecithans from various sources.

#### HERZIG AND MEYER'S DETERMINATION OF METHYL ATTACHED TO NITROGEN

The apparatus consists of a double glass bulb, 4 cm. wide at the largest diameter and 2½ cm. at the narrowest diameter, and 12 cm. high. The bulb (a) is connected to (b) by a glass tube which runs to the bottom of (b). The double bulbs are placed in an iron sand bath with double bottom and a partition (cd) so that (a) can be heated in sand, while (b) remains comparatively cool. C is a flask for catching the distillation products from the bulbs,



<sup>10</sup> *Op. cit.*, p. 128.

<sup>11</sup> *Ibid.*, p. 149.

<sup>12</sup> *Op. cit.*, p. 50.

<sup>13</sup> HERZIG AND MEYER, *Monatshefte für Chemie*, Vol. XV, p. 613.

at *H*, to enter the condenser *D* and keep it at the proper temperature. By regulating the flow of the water and the height of the flame, the temperature of the water can easily be kept within the required limits. *F* are Geissler bulbs for absorbing everything but the methyl iodide, which is absorbed in *K* and *L*. The analysis is carried on as follows: 0.2 g. of the substance to be analyzed is placed in (*a*) with 2 g. of dry ammonium iodide and enough hydriodic acid (sp. gr. 1.6) to half fill the lower bulb. In (*b*) is placed 1 g. of ammonium iodide. The part *A* of the sand bath is filled with sand and a thermometer reading to 360° C. placed in the sand. A stream of dry CO<sub>2</sub> is allowed to enter at *M*, and when all the air is displaced, a triple burner is lighted under the sand bath. In the meanwhile the water must be started and kept running through the condenser *D* at a temperature of from 40° to 50° C. As the temperature of 200° C. is reached in the sand bath, methyl iodide begins to split off and is carried over by the CO<sub>2</sub> mixed with hydriodic acid and iodine. Most of the iodine and hydriodic acid is condensed at *D* and collected in *C*. Some passes over and is absorbed in *F*, which contains the following solution:

Sodium carbonate	-	-	-	-	1 part
Potassium arsenite	-	-	-	-	1 part
Water	-	-	-	-	10 parts

The methyl iodide passes on and is collected in *K*, which contains 2 g. of silver nitrate dissolved in 5 c.c. water and 45 c.c. absolute alcohol. The methyl iodide dissolves in the alcohol, and is decomposed by the silver nitrate with the formation of silver iodide. After some time the temperature in the sand bath gradually rises to 240° C., and after a little while longer methyl iodide ceases to come over, as can be seen by the liquid in *K* becoming perfectly clear. *L*, which also contains silver nitrate, is used merely as a guard. The solution can be removed at this point, and the silver iodide collected corresponds to all the kephalin and one methyl group of the lecithin. Fresh silver nitrate is placed in *K*, another burner placed under the sand bath, and the temperature raised to 300° C. The remaining two methyl groups of lecithin come over, while kephalin gives off no more, or only a trace, of methyl iodide. The second part of the sand bath, *B*, is now filled with sand and heated to 300° to decompose anything which may have escaped previous heating. The two alcoholic solutions containing the silver iodide are diluted with much water and warmed for several hours on a steam bath to remove alcohol. Strong nitric acid is then added, and the silver iodide filtered into a Gooch crucible and weighed. In case we are not dealing with a mixture of lecithans, all the silver iodide can be weighed in one portion.

#### PREPARATION AND ANALYSES OF VARIOUS LECITHANS

*Egg lecithin*.—The yolks of ten eggs are allowed to stand with 600 c.c. ether over night, 1 liter alcohol added, the solution filtered and evaporated on water bath. The residue is dissolved in 200 c.c. cold ether and 1 liter acetone added. The precipitated

lecithin is treated over night with 1 liter cold alcohol, the solution filtered and evaporated. The residue is once more dissolved in ether, precipitated with acetone, and dried over sulphuric acid in a vacuum desiccator.

I.<sup>14</sup> 0.8113 g. of the substance gave 0.1136 g.  $\text{Mg}_2\text{P}_2\text{O}_7$ ; *i. e.*, 3.91 per cent. P.

II. 0.9150 g. of the substance gave 0.1278 g.  $\text{Mg}_2\text{P}_2\text{O}_7$ ; *i. e.*, 3.90 per cent. P.

III. 0.330 g. of the substance gave 0.3001 g. AgI; *i. e.*, 5.80 per cent.  $\text{CH}_3$ .

IV. 0.325 g. of the substance gave 0.2960 g. AgI; *i. e.*, 5.81 per cent.  $\text{CH}_3$ .

V. 0.320 g. of the substance gave, below  $240^\circ\text{C}$ ., 0.0760 g. AgI; *i. e.*, 153 per cent.  $\text{CH}_3$ . The methyl iodide given off above that temperature was lost on account of an accident. In III and IV the methyl iodide came over at  $220^\circ\text{C}$ . and  $300^\circ\text{C}$ . The two portions were not separated.

Phosphorus as 3.97 calculated for	Found		
	III	IV	V
3 $\text{CH}_3$ : 5.66	5.80	5.81	
1 $\text{CH}_3$ : 1.88			1.53

*Brain lecithin and kephalin.*—One kilo sheep's brains is minced in a meat-chopper and freed from water and extractives by boiling with 1 kilo acetone for eight hours. The solution is filtered cold, and the remaining acetone removed from the brains by gentle heating at  $50^\circ\text{C}$ . Seven hundred c.c. cold ether are now added and allowed to stand for three days, the solution is filtered, and another portion of ether added, and again allowed to stand. The ether filtrates are united and slowly evaporated to one-fourth their original volume in a tall beaker. The solution is then carefully removed by means of a pipette from the white precipitate, which has settled to the bottom, and 1.5 kilo alcohol is added to the solution.

*Kephalin.*—The precipitated kephalin is extracted five times with boiling alcohol, dissolved in ether, precipitated with acetone, again dissolved in ether, and allowed to settle in a long, narrow, closed test-tube. The clear ether solution is removed by decantation, evaporated, and the residue recrystallized twice from hot acetic ether. The resulting kephalin is very hygroscopic and must be dried over sulphuric acid for analysis. It agrees perfectly in all its properties with the kephalin described by Thudichum (p. 127). On analysis it gave the following results:

I. 0.2469 g. of the substance gave 0.5388 g.  $\text{CO}_2$  and 0.2159 g.  $\text{H}_2\text{O}$ ; *i. e.*, 59.5 per cent. C and 9.7 per cent. H.

II. 0.415 g. of the substance neutralized 5.2 c.c.  $\frac{1}{100}$  acid; *i. e.*, 1.78 per cent. N.

III. 0.9644 g. of the substance gave 0.1330 g.  $\text{Mg}_2\text{P}_2\text{O}_7$ ; *i. e.*, 3.85 per cent. P.

IV. 0.7235 g. of the substance gave 0.0990 g.  $\text{Mg}_2\text{P}_2\text{O}_7$ ; *i. e.*, 3.82 per cent. P.

V. 0.3488 g. of the substance gave, below  $240^\circ\text{C}$ ., 0.0945 g. AgI; *i. e.*, 1.73 per cent.  $\text{CH}_3$ .

VI. 0.490 g. of the substance gave, below  $240^\circ\text{C}$ ., 0.1312 g. AgI; *i. e.*, 1.71 per cent.  $\text{CH}_3$ .

<sup>14</sup>For phosphorus determinations the very excellent method of Neumann was used (*Engelmann's Archiv für Physiologie*, 1900, p. 159).

VII. 0.225 g. of the substance gave, below 300° C., 0.0636 g. AgI; *i. e.*, 1.80 per cent. CH<sub>3</sub>.

VIII. 0.748 g. of the substance were burned with a mixture of nitric and sulphuric acid, the solution diluted, neutralized with 50 g. barium hydrate to remove sulphates and phosphates, the barium removed with sulphuric acid, and the solution evaporated. The ignited residue weighed 0.030 g. Some of this must have come as an impurity from the barium hydrate. In any case, the amount is not sufficient to account for any more than an impurity. Thudichum<sup>18</sup> has also found his kephalin to contain small amounts of inorganic substances as impurities. My analyses agree fairly well with Thudichum's (p. 132):

Thudichum: C, 60.0; H, 9.38; N, 1.68; P, 4.27

My results: C, 59.5; H, 9.7; N, 1.78; P, 3.84

The methyl groups correspond very closely to one methyl for one nitrogen. That this is not due to the presence of lecithin as an impurity is shown by the fact that the methyl is all split off below 240° C.

Phosphorus as 3.83 calculated for	Found			
	II	V	VI	VII
1 CH <sub>3</sub> :		1.73	1.71	1.80
1 N:	1.73			

*Lecithin*.—The original alcohol-ether filtrate from which the kephalin has been removed by filtration is evaporated to dryness, and the residue dissolved in ether and freed from cholesterol by precipitation with acetone. The precipitated lecithin is treated with a large quantity of cold alcohol for some time, and the solution filtered and evaporated. The resulting lecithin should dissolve readily and completely in cold alcohol or ether. If this is not the case, the extraction with cold alcohol must be repeated. Finally, the lecithin is dissolved in hot acetic ether and allowed to separate out by cooling, dried over sulphuric acid, and analyzed.

I. 0.2420 g. of the substance gave 0.5683 g. CO<sub>2</sub> and 0.2420 g. H<sub>2</sub>O; *i. e.*, 64.04 per cent. C.; 10.4 H.

II. 0.942 g. of the substance neutralized 12.1 c.c.  $\frac{N}{10}$  acid; *i. e.*, 1.8 per cent N.

III. 0.677 g. of the substance gave 0.0919 g. Mg<sub>2</sub>P<sub>2</sub>O<sub>7</sub>; *i. e.*, 3.79 per cent. P.

IV. 0.815 g. of the substance gave 0.1109 g. Mg<sub>2</sub>P<sub>2</sub>O<sub>7</sub>; *i. e.*, 3.80 per cent. P.

V. 0.3975 g. of the substance gave 0.3156 g. AgI; *i. e.*, 5.1 per cent. CH<sub>3</sub>.

VI. 0.235 g. of the substance gave 0.1853 g. AgI; *i. e.*, 5.03 per cent. CH<sub>3</sub>.

My lecithin agrees fairly well in its properties with the one isolated by Thudichum (p. 116). The results of the methyl-group determination show as good an agreement with the theory as can be expected from a compound so difficult to purify.

Phosphorus as 3.8 calculated for	Found		
	III	V	VI
3 CH <sub>3</sub> :		5.1	5.03
1 N:	1.72	1.8	

<sup>18</sup> *Op. cit.*, p. 130.

*Lecithans from yeast.*—Twenty yeast cakes are mixed well with one liter of alcohol in a flask and boiled for eight hours, filtered, the yeast allowed to stand with ether over night, filtered, the filtrates united and evaporated. The residue is extracted with cold ether, three times the volume of acetone added, and the resulting precipitate dried *in vacuo* over sulphuric acid, as it is not sufficient for further purification. The substance thus obtained resembles kephalin in that it darkens rapidly on exposure to the air and is precipitated from ether solution by alcohol. The analysis gave the following result:

I. 0.220 g. of the substance gave 0.0286 g.  $\text{Mg}_2\text{P}_2\text{O}_7$ ; *i. e.*, 3.63 per cent. P.

II. 0.165 g. of the substance gave 0.0626 g.  $\text{AgI}$ ; *i. e.*, 2.42 per cent.  $\text{CH}_3$ .

The methyl iodide was split up mostly at  $240^\circ \text{C}$ ., but some more was obtained on raising the temperature to  $300^\circ \text{C}$ .

Phosphorus as 3.63 calculated for	Found II
1 $\text{CH}_3$ : 1.76	2.42
2 $\text{CH}_3$ : 3.51	

The amount of methyl is not sufficient to account for two groups, and as the compound was not especially freed from lecithin, it seems reasonable to account for the extra methyl as due to an impurity of lecithin which would amount to about 11 per cent. The main bulk of the body is, therefore, kephalin, which agrees well with the other observations.

The results so far obtained with the Herzig and Meyer methyl group determine, then, that the two principal classes of lecithans differ, not only in the fatty acid group, but also in the complex which contains the nitrogen. Very striking is the fact that kephalin occurs only in living cells, such as the nerve or yeast cell, and is not found in the egg, which consists mostly of stored food material. Kephalin may possibly be an intermediary product in the decomposition of lecithin. The low amount of carbon and the correspondingly large amount of oxygen would indicate an addition of oxygen in the oleic-acid radical of the lecithin—a hypothesis which leaves unexplained the fact that kephalin is, if anything, more unsaturated than lecithin, judging by the relative amounts of iodine absorbed. At any rate, there is at present not enough known about the nature of kephalinic acid to trace its origin to oleic acid. As far as the nitrogen complex is concerned, it is possible that two methyl groups have been split off, leaving a mono-methyl oxæthylamin. Thudichum<sup>16</sup> has, indeed, isolated from his kephalin a base which contains less methyl groups, but he has also mentioned the presence of neurin. My results, however, show conclusively that neurin can be present only as an impurity. The decomposition of twenty grams of kephalin with barium hydrate yielded only 0.2 g. of a platinum salt, which corresponded, on analysis, to something between a mono- or a di-methyl oxæthylamin. The investigation of this interesting relation will be continued, and the methyl-group determination described above will undoubtedly be of value in following out the quantitative relation of lecithin to kephalin under various

<sup>16</sup> *Op. cit.*, p. 147.

conditions in the living cell. The other lecithans, such as the myelins, paramyelins, and amidomyelins, isolated by Thudichum from brain tissues, do not seem to occur in any large quantity and have not as yet been investigated.

#### PHYSIOLOGICAL PROPERTIES

Substances of such importance to the cell as the lecithans must possess some value as foods. The action of the digestive ferments is therefore of especial importance, as neurin, which is formed by the decomposition, has been shown by Halliburton<sup>17</sup> to have a decided effect on blood pressure. A. Bókay,<sup>18</sup> under the direction of Hoppe Seyler, investigated this problem and found that lipase will split egg lecithin into glycerophosphoric acid, fatty acids, and neurin. The three splitting products must, however, be immediately absorbed and resynthesized, as they are not found in the urine or fæces, and a meal containing considerable lecithin has never been known to cause any bad effects. The results on the metabolism of the injection of lecithin into the circulation are as yet in too unsatisfactory a state to be discussed. The patenting<sup>19</sup> of brain preparations for medical purposes seems, therefore, especially premature.

For completeness' sake a number of facts have been mentioned in this paper which have been known for a long time. The new facts are as follows:

1. The word "lecithan" is to be used as a group name, including such compounds as egg lecithin, brain lecithin, kephalin, myelin, paramyelin, etc.
2. The emulsion formed by the lecithans may be the substratum in which the reactions of the cell take place. The precipitation of this emulsion by divalent kations is prevented by univalent and trivalent kations, as far as investigated, and this observation may furnish an explanation of the changes brought about by electrolytes in the living cell.
3. Kephalin is found only in the living cell, and may be an intermediary product in the metabolism of lecithin.
4. An accurate method for determining lecithin and kephalin quantitatively.

<sup>17</sup> *Op. cit.*, p. 55.

<sup>18</sup> A. BÓKAY, *Hoppe Seyler's Zeitschrift für physiologische Chemie* (1877), Vol. I, p. 157.

<sup>19</sup> C. ZERBE, *Chemiker-Zeitung* (1902), Vol. XXVI, p. 17; Deutsches Reichs-Patent, 127357.

## **ABSORPTION OF LIQUIDS BY ANIMAL TISSUES**





# A CONTRIBUTION TO THE PHYSICAL ANALYSIS OF THE PHENOMENA OF ABSORPTION OF LIQUIDS BY ANIMAL TISSUES

RALPH W. WEBSTER

## I. INTRODUCTION

THE following paper is, as the title indicates, intended to be a contribution to the physical analysis of phenomena of absorption by living tissues. That the older experiments on absorption could not lead to any satisfactory explanation of the processes involved seems evident from the fact that only recently has there been discovered one of the most fundamental theories concerning the exchange of liquids separated by membranes, more or less semi-permeable. Only such papers can be expected to throw a light on this subject as take cognizance of this theory of osmotic pressure.

Van't Hoff, applying certain facts brought out by Traube and Pfeffer regarding the influence of semi-permeable membranes upon processes of osmosis, showed that substances in solution obey the ordinary laws of gases, as brought forth by Boyle, Henry, Gay-Lussac, and Avogadro. In consequence of this similarity between gases and substances in solution, the latter will exert a pressure upon the walls of a containing vessel equal to the pressure which the dissolved substance would exert were it present in the gaseous form under the same conditions of temperature and molecular aggregation. Whether this pressure, which van't Hoff calls "osmotic pressure," be due to the impacts of the dissolved particles against the walls of the containing vessel, as the kinetic theory of gases would demand, or whether it be an expression of the attraction of the dissolved particles for water, concerns us, in these experiments, only in so far as our work has to do with the dynamics of the process of absorption.

From these facts it is evident, as van't Hoff shows, that the pressure of a substance in solution depends both upon the concentration of the substance and upon the temperature at which the observation is made. By the concentration we mean, not the number of molecules, as such, contained in a definite amount of the solvent, but, rather the total number of "active" particles contained in a definite (usually 1 liter) amount of solvent. This fact was made clear by the endeavor to collate the pressures of various salt solutions with those of organic substances, or, in other words, of electrolytes with those of non-electrolytes. Clausius had shown that the molecules of substances conducting electricity, viz., of electrolytes, are dissociated into ions, which have a movement independent of one another. Arrhenius, in his work on *Dissociation of Substances Dissolved in Water*, advanced the hypothesis that the molecules of substances in solution suffer a dissociation into their electrically-charged ions (an ion being considered as an atom, or group of atoms, carrying an electric charge + or -, according

to the electric nature of the element). This conception of Arrhenius gave the new idea that the dissociation was due to the mere dissolving of the electrolyte in the water, and not, as Clausius had claimed, to the action of the electric current. This electrolytic dissociation of substances in watery solution is by no means complete at every concentration, but increases with the dilution until, at infinity dilution, a complete dissociation of the molecules into their respective ions takes place. We have, therefore, in a watery solution of an electrolyte two kinds of molecules—the active (electrically dissociated), and the inactive (non-dissociated). Inasmuch as the ions of the dissociated molecules each exert pressure, we may readily understand how the osmotic pressure of a substance in watery solution will show a much higher value as the degree of dissociation becomes greater.

The bearing of the theory of osmotic pressure upon phenomena of absorption is easily seen to be of the first magnitude. If a substance in watery solution be separated from the pure solvent by a membrane permeable to the solvent, but not to the solute, we have the conditions necessary for the solving of problems of osmosis. In such a case, as van't Hoff showed, water will pass into the solution, and, after a time, will establish a condition of equilibrium due to the pressure of the water which enters in minimal quantities. Of course the water, under such circumstances, does not give rise to the osmotic pressure measurable by a manometer, inasmuch as it is present on both sides of a membrane permeable to it. The pressure is, in this case, due solely to the dissolved particles, and may be explained by the kinetic theory, or the water-attraction of the parts dissolved.

If, instead of a membrane strictly impermeable to the dissolved particles, we have one which allows the passage of some, at least, of the dissolved particles we have a slightly different condition of affairs. In such a case the osmotic pressure will be a minimum and the process will resolve itself into one of diffusion, as the membrane being permeable to the solvent and solute, will not in any way hinder the diffusion process. However, if we add to such a condition the further complexity of two solutions separated by a membrane, freely permeable to the solvent and difficultly so for the solute, we have the conditions as they exist in the various cells of the body.

Experiments on absorption of liquids, or, in other words, upon processes involved when the conditions stated above exist, have been made on organized as well as unorganized material. The tissues chiefly involved in such work have been red-blood corpuscles, muscles, and intestines; while the unorganized material has been that known under the general name of colloid matter, including, here, gelatine, albumin, sodium-oleate, silicon-dioxide, etc. In the present work I have confined myself chiefly to the effects of solutions of various electrolytes and non-electrolytes upon absorption by muscular tissue. However, a few introductory experiments upon red blood-corpuscles were made, as it had been shown by various workers that absorption by these cells obeys the laws of osmotic pressure to a large extent, while my results on muscular tissue seem to show that variations in osmotic pressure cannot account, entirely, for phenomena noted in the latter case.

## II. EXPERIMENTS ON RED BLOOD-CORPUSCLES

It seems to be generally agreed that these cells may be regarded as systems surrounded by semi-permeable membranes. If two solutions of different osmotic pressure be separated by a more or less elastic, semi-permeable membrane, an attempt is made to equalize the pressure on each side of this membrane, with the result that phenomena occur of swelling or of shrinking of the cell in question.

Donders, Hamburger, Koeppe, Gryns, Hedin, Overton, Roth, and others have concluded, from their work on these corpuscles, that the phenomena mentioned above depend on the difference of osmotic pressure between the solution outside and that inside the corpuscular membrane, which membrane may be regarded as a thickening of the protoplasm of the corpuscle. So convinced is Koeppe of the rôle of osmotic pressure in life phenomena that he gives expression to the generality: "We cannot imagine a single phenomenon in the living organism in which osmotic pressure may not have a share."

One of the most important points to be considered in this discussion of effects of difference in osmotic pressure is that of the permeability of the corpuscle to the molecules, or ions, of the substances investigated. If it can be shown that the corpuscle be permeable to these particles, then, of course, the laws of diffusion replace those of osmotic pressure. The process, in this latter case, would then resolve itself simply into a discussion of the relative rate of diffusion of the various particles in question.

Certain facts and experiments show, rather conclusively, that the corpuscular membrane is to be regarded as a semi-permeable membrane, permeable to the molecules of water (and to ions, or molecules, of certain inorganic and organic substances) but impermeable, to a large extent, to substances in solution both as regards the solution inside and that outside the membrane. As Koeppe states, the red corpuscles contain no sodium chloride, while the serum contains 5.546 gms. in 1,000 gms. On the other hand, the corpuscles contain 3.679 gms. of potassium chloride, while the serum contains only .39 gms. in 1,000 gms. These facts, in themselves, show that the corpuscle is impermeable to the ions of the salts in question, else an equilibrium would be established on both sides of a permeable membrane. Gryns, Eykman, Hedin, and Oker-Blom have carried out extensive series of experiments to show the relative permeability of the corpuscle to various substances. Gryns, in his work on *Influence of Dissolved Substances upon Red Blood-Corpuscles in Connection with Phenomena of Osmosis and Diffusion*, gives a tabular list of substances to which the corpuscle is permeable and impermeable. Among these we find salts of metals, certain  $\text{NH}_4$  salts, such as sulphate, nitrate, phosphate, and tartrate, glycocoll, sugar, etc., to be incapable of penetrating the corpuscle; while other  $\text{NH}_4$  salts, such as chloride, bromide, and oxalate, alcohol, glycerine, urea, etc., easily penetrate this cell. Hedin and Oker-Blom, working with methods different from those of Gryns, confirm his results.

A fact of the utmost importance in this discussion of the permeability of the corpuscular membrane, as well as of any other membrane, to different substances is the following: A removal of free ions from a solution is possible only when ions of oppo-

site electric charges are removed together, because the electric charges of the ions hinder the free movement from the solution. Therefore, if a semi-permeable membrane is impermeable to one ion of a molecule it is impermeable to the other, for, if this were not true, a separation of positive or negative electricity would take place.

In solutions, therefore, of any of these substances to which the corpuscular membrane is impermeable, phenomena of swelling or of shrinking occur if the osmotic pressure of the outside solution be less, or more, than that of the solution within this membrane. This osmotic value of the solution within the corpuscular membrane varies, according to Hamburger, between that of a .75 per cent. NaCl solution and that of a .9 per cent. NaCl solution.

If, now, we place the corpuscles in solutions of substances to which they are permeable, osmotic pressure effects play no rôle whatever, even though the concentration of the outside solution be of higher, equal, or lower value than that inside the membrane. We find that, in these latter cases, phenomena of swelling or shrinking do not occur. As Hedin points out, the blood corpuscles do not, under such circumstances, obey the laws of osmotic pressure, and, as a result, they give up their haemoglobin to the outside solution.

Hamburger, in his earlier experiments, had attempted to apply a method based upon that of De Vries's classic work on plasmolysis in plants. He used, in these experiments, the point at which the corpuscle began to lose its haemoglobin as his isosmotic point. He maintained that the corpuscles were, to a high degree, permeable to the salts in question. As a result of this, he says, an equilibrium is established between the osmotic pressure on each side of the membrane. These ideas are, as shown above, totally at variance with the theory of osmotic pressure as advanced by van't Hoff.

In his work Oker-Blom advanced the idea that entirely erroneous conceptions of the osmotic equivalents of the corpuscles must creep in by use of solutions of salts in water. He advocates the use of solutions in serum, inasmuch as the variation introduced in osmotic pressure is, then, referable to the salt added, without considering the consequent lowering of the concentration of the blood on addition of a watery solution of the salt. He shows that, in previous work along these lines, there has always been a question of reciprocal action between the substances present in the animal cells and those with which the cells are brought into contact. In view of such conditions, he says, only the total osmotic pressure of the fluids under observation has been considered, while, from his work, it seems quite evident that the entrance of a substance into a red blood-corpuscle is, clearly, under the influence of the partial osmotic pressure of the substance added, inasmuch as no great increase in total osmotic pressure has been caused by this addition.

My own experiments, few in number, were planned simply with the idea of showing, if possible, that we are dealing, in the case of the red corpuscle, with osmotic pressure effects to a large extent. These experiments have no claim to originality, but are confirmatory of the work previously mentioned. The method followed in this work is a slight modification of Hedin's and Koeppe's hæmatokrit method. Mixtures

were made of defibrinated blood and of isosmotic salt solutions in various dilutions. In some cases equal parts of each (blood and salt solution isosmotic with blood) were taken. In other cases, 2, 3, 5, and 10 parts of solution to 1 of blood were used. These mixtures were placed in dishes containing, approximately, 25 c.c., and allowed to stand for intervals of 1, 3, 6, and 24 hours. At the end of each interval readings were taken by the hæmatokrit method, using one revolution per second for 3 minutes. By this method the percentage relation of corpuscles to serum was obtained. These experiments can have no further value than a comparative one, inasmuch as the osmotic pressure of the mixtures was not determined, and thus the concentration of the solution acting on the corpuscle was unknown. However, as solutions of salts isosmotic with the blood serum and, therefore, with one another were used, we may readily show whether osmotic pressure differences account for phenomena noted or whether we are dealing with specific ionic effects. Below will be found a table embodying the results of these experiments:

TABLE I

Substance	Concentration	BLOOD DILUTION		Per Cent. Vol. of Corpuscles in 3 Hours	Substance	Concentration	BLOOD DILUTION		Per Cent. Vol. of Corpuscles in 3 Hours
		Solution	Blood				Solution	Blood	
NaCl ....	$\frac{1}{2}$ m	10	5	10	KCl....	$\frac{1}{2}$ m	10	5	11
NaCl ....	$\frac{1}{2}$ m	10	10	19	KCl....	$\frac{1}{2}$ m	10	10	22
NaCl ....	m	10	5	8	KCl....	$\frac{1}{2}$ m	5	5	17
NaCl ....	m	10	10	15	KCl....	$\frac{1}{2}$ m	10	5	10
NaCl ....	$\frac{1}{2}$ m	10	5	9	KCl....	$\frac{1}{2}$ m	15	5	7
NaCl ....	$\frac{1}{2}$ m	10	10	20	KCl....	$\frac{1}{2}$ m	10	10	25
NaCl ....	$\frac{1}{2}$ m	10	5	12	KCl....	$\frac{1}{2}$ m	60	20	21
NaCl ....	$\frac{1}{2}$ m	10	10	22	KCl....	$\frac{1}{4}$ m	10	5	dissolution
NaCl ....	$\frac{1}{2}$ m	5	5	17	KCl....	$\frac{1}{4}$ m	10	10	32
NaCl ....	$\frac{1}{2}$ m	10	5	11	KCl....	$\frac{1}{4}$ m	10	5	dissolution
NaCl ....	$\frac{1}{2}$ m	15	5	9	KCl....	$\frac{1}{4}$ m	10	10	7
NaCl ....	$\frac{1}{2}$ m	10	10	28	CaCl <sub>2</sub> ...	$\frac{1}{2}$ m	20	20	33
NaCl ....	$\frac{1}{2}$ m	20	20	42	CaCl <sub>2</sub> ...	$\frac{1}{2}$ m	60	20	12
NaCl ....	$\frac{1}{2}$ m	60	10	10	CaCl <sub>2</sub> ...	$\frac{1}{2}$ m	60	10	8
NaCl ....	$\frac{1}{2}$ m	60	20	19	CaCl <sub>2</sub> ...	$\frac{1}{2}$ m	5	5	15
NaCl ....	$\frac{1}{4}$ m	10	5	dissolution	CaCl <sub>2</sub> ...	$\frac{1}{2}$ m	10	5	12
NaCl ....	$\frac{1}{4}$ m	10	10	30	CaCl <sub>2</sub> ...	$\frac{1}{2}$ m	15	5	8
NaCl ....	$\frac{1}{4}$ m	10	5	dissolution	NH <sub>4</sub> Cl.	$\frac{1}{2}$ m	5	5	dissolution
NaCl ....	$\frac{1}{4}$ m	10	10	8	NH <sub>4</sub> Cl.	$\frac{1}{2}$ m	10	5	dissolution
KCl ....	$\frac{1}{2}$ m	10	5	6	NH <sub>4</sub> Cl.	$\frac{1}{2}$ m	15	5	dissolution
KCl ....	$\frac{1}{2}$ m	10	10	16	NH <sub>4</sub> Cl.	$\frac{1}{2}$ m	10	10	dissolution
KCl ....	m	10	5	5	Urea ...	.2286 m	5	5	dissolution
KCl ....	m	10	10	12	Urea ...	.2286 m	10	5	dissolution
KCl ....	$\frac{1}{2}$ m	10	5	9	Urea ...	.2286 m	15	5	dissolution
KCl ....	$\frac{1}{2}$ m	10	10	18	Urea ...	.2286 m	10	10	dissolution

A study of this table shows two facts quite clearly. The first is, that we have two classes of substances represented here. The one class, to which the corpuscular membrane is permeable, embraces NH<sub>4</sub>Cl and urea; the other class, to which the

membrane is impermeable, includes NaCl, KCl, and  $\text{CaCl}_2$ . The second fact brought out by this table is, that the phenomena of swelling and shrinkage of the corpuscles, when placed in salt solutions of various dilutions, are under the influence of osmotic pressure differences and not under the influence of specific ionic effects.

These experiments confirm in a simple way the results of Gryns and Oker-Blom, by showing the permeability of the corpuscle to various substances and the impermeability to others.

### III. EXPERIMENTS ON MUSCULAR TISSUE

The work on the influence of osmotic pressure upon phenomena of swelling in muscle has been largely carried out by Professor Loeb and his pupils. He found, in his work on the effects of ions, that the addition of a small amount of a dilute acid or alkali to a physiological salt solution caused a muscle, immersed in such a solution, to take up water, or, in other words, to gain in weight to a considerable extent. Further investigation showed that this effect was due to the number or concentration of free H ions in the former and of free OH ions in the latter case. Miss Cooke, working under the direction of Professor Loeb, found that a muscle immersed in  $\frac{1}{8}$  m NaCl for some time (eighteen hours) neither gained nor lost in weight. She therefore assumed that such a solution was isosmotic with the muscle-plasma. This conclusion is open to some of the objections raised against the work on red corpuscles, with the addition that here we are dealing with a membrane, the sarcolemma, which allows the passage of ions in both directions, and hence does not as fully obey the laws of osmotic pressure as do the corpuscles. As will be shown later, the sarcolemma is permeable to Na and K ions, and hence we should expect the same result with isosmotic solutions of Na and K salts. This, however, is not the case, as Loeb himself points out in his further work along this line. He shows that a muscle immersed in  $\frac{1}{8}$  m NaCl for eighteen hours gains only slightly (5 per cent.) in weight; one immersed in  $\frac{1}{8}$  m KCl gains 50 per cent., while in  $\frac{1}{10}$  m  $\text{CaCl}_2$  it loses 20 per cent. He advances as explanation the hypothesis that we have to do with a chemical combination of the ions of the salt with the proteid molecule, giving us what he terms "ion-proteids," which he assumes show similar reactions, especially as regards their fluid absorbing power to those of soaps.

From the study of the work of Hofmeister, Lewith, van Bemmelen, Pauli, and Hardy on effects of salts upon colloids, we obtain a much clearer insight into the properties, both physical and chemical, of the body-colloids. Hofmeister attempts to show that the albumin and globulin precipitating power of salts is due to their dehydrating power, giving as his reasons for this view (1), the precipitating power remains practically the same for action of different salts on various colloids (the degree of dissociation of the salts being a great factor); (2), this power goes parallel to other physical and chemical properties of salts which are dependent on dehydrating power. In his work on the phenomena of swelling he calls attention to the fact that we must take cognizance of three forces, viz.: capillary imbibition, endosmotic imbibition, and

molecular imbibition. Through these forces, he is led to believe, the absorption of water and of salts is controlled, it being quite evident that the absorption of water and salts goes on quite independently the one of the other.

Van Bemmelen, in his work on absorption by colloids, has shown that inorganic colloids, such as  $\text{SiO}_2$ ,  $\text{Fe}(\text{OH})_3$ , etc., have quite a similarity to organic colloids. The absorption of water and of salts from solutions is dependent on the nature of the substance in solution as well as on the concentration of this substance. Absorption by inorganic colloids, he states, depends on several factors, (1) structure of colloid, (2) modification brought about in this structure through gel-formation, heat, etc., (3) vapor-tension (osmotic pressure) of fluid to be absorbed, (4) temperature, (5) kind of solvent. Great interest attaches itself to his work on the conversion of a hydrosol into a hydrogel. He declares that salts (with strong acids) of trivalent metals have the strongest coagulating power, then follow in turn the salts of bivalent and univalent elements. This fact has been elaborated by Hardy into the law that "the coagulating power of salts of elements increases as the 2nd, 3rd . . . power of the valence of that element." In this connection Hardy states that, in general, electrolytes have an effect while non-electrolytes have none; a statement quite in harmony with those made by Loeb, Lingle, Moore, Mathews, and others concerning the action of electrolytes and non-electrolytes on organic colloids.

In order to test the conclusions of the various workers and to show, further, the action of various ions as well as the rôle of osmotic pressure in the phenomena of absorption of liquids by muscular tissue, this work was undertaken at the suggestion of Professor Loeb.

#### IV. MATERIAL AND METHOD

Owing to the fact that it is readily accessible, that any variations in its condition may be easily controlled, and that experiments with it may be accurately carried out, the gastrocnemius muscle of the frog was selected as the research material for these experiments. The muscle is removed from the leg, care being taken not to injure the muscle substance, not to use for experimentation muscles which have been very active previous to removal, and to exclude those muscles which show bruises or hemorrhagic areas. After being carefully dried with sheets of filter-paper, the muscle is placed between watch-glasses and accurately weighed. Thus weighed the muscle is placed in a dish containing approximately 25 c.c. of the solution of the substance whose action is under investigation. The concentration of the solutions varied within rather wide limits, inasmuch as it seemed desirable to ascertain the effects of various concentrations of the same substance, as well as the same concentration of various substances. The working basis of the concentration was made that of  $\frac{1}{8}$  m NaCl, as this had been previously shown by Miss Cooke to be isosmotic with the muscle-plasma. The concentrations of the various solutions were graded, starting with  $\frac{5}{4}$  m., following with m,  $\frac{1}{2}$  m,  $\frac{1}{4}$  m,  $\frac{1}{8}$  m,  $\frac{1}{16}$  m, and ending with  $\frac{1}{32}$  m. This range seemed necessary in order to give ample scope to the study of hyper-, iso-, and hypo-tonic solutions. In the later experiments only solutions isosmotic with  $\frac{1}{8}$  m NaCl were used, as it appeared possible,



by this means, to arrive at more definite conclusions concerning the effect of osmotic pressure. In calculating the strength of a solution which shall be isosmotic with a known solution, it is necessary only to make use of the simple formula:

$$y = \frac{P}{22.35 (1 + (n-1) a)} \text{ in which}$$

$P$  = osmotic pressure of known solution.

$y$  = strength of unknown solution in terms of normal solution.

$m = n, 2n, 3n$ , etc., according to basicity of salt.

$n$  = number of ions into which the molecule of salt is dissociated.

$a$  = degree of dissociation of solution of same molar concentration as known solution.

After remaining in such solutions for intervals of one, three, six, and twenty-four hours, the muscle is taken out, carefully dried, as before, and weighed. In the drying of the muscle with filter paper, two errors are prone to creep into the work and should be guarded against. The first of these is a positive one and consists in not removing all of the fluid adhering to the surface. The second is a negative one and consists in exerting undue pressure on the muscle, thereby drying out the surface layer of muscle, and causing an increase of pressure upon the inner layers. This latter error is greater, of course, the smaller the muscle, inasmuch as we have a much larger surface in proportion to the total mass of muscle. The percentage gain or loss in weight of the muscle (as ascertained by comparing the weight before immersion in the solution, with the weight after removal from the same solution), gives the absorption. In the experiments detailed here the gain or loss is interpreted as meaning an increase or decrease in the amount of water in the muscle, although it is more than probable that the change is due to interchange of ions through the sarcolemma and an ultimate establishment of equilibrium between partial osmotic pressures on both sides of this membrane.

#### V. EFFECTS OF NON-ELECTROLYTES

We understand by non-electrolyte any substance which exists in solution in the molecular and not in the ionic form. Such substances, when in solution, do not conduct electricity and do not show irregularities in lowering of the vapor tension of the solution. They obey the laws of osmotic pressure, however, and therefore give us opportunity of studying the osmotic effects in absorption phenomena, although the ionic effects are excluded by their use. The nonelectrolytes used in these experiments were water, cane sugar, and urea. The concentrations were, except naturally in case of water, made isosmotic with the various NaCl solutions to be used later.

*a. Absorption from Redistilled Water.*—A gastrocnemius muscle placed in redistilled water goes through the following phases of absorption:

Absorption			
1 h.	3 h.	6 h.	24 h.
+ 34	+ 56	+ 72	+ 67

These results show, beyond a doubt, that in this case we are dealing with a purely osmotic phenomenon. The natural result of placing a solution of salts (the

muscle-plasma) upon one side of a membrane partially permeable (as will be shown later) to these salts, and, on the other side of this membrane, pure water to which the membrane is more or less freely permeable, is a rapid passage of water into the salt solution and a very slow passage of ions into the water. As the time of the experiment is extended, we find that a point is reached when the outside concentration of salt may be greater than that inside. In consequence of this condition water passes from the muscle into the solution. Possibly the osmotic pressure within the muscle may be increased by the production of sarco-lactic acid, in which case the steady increase in absorption may be accounted for. Hofmeister states that a muscle immersed in water absorbs a certain amount, which cannot exceed a limit known as the "maximum of swelling," which limit is dependent on the amount of (1) capillary imbibition, (2) osmosis, and (3) adsorption. Our results show that a large part of the absorption from water is due to direct endosmotic imbibition.

b. *Absorption from Cane Sugar Solutions.*—The absorption noted when a muscle is placed in solutions of various concentrations of cane sugar is as follows:

TABLE II

Concentration	ISOSMOTIC	ABSORPTION			
	NaCl Sol.	1 h.	3 h.	6h.	24 h.
1.6745 m .....	m	-40	-53	-57	-53
.8667 m .....	$\frac{1}{2}$ m	-27	-35	-35	-22
.4465 m .....	$\frac{1}{4}$ m	-12	-12	-12	-2
.2286 m .....	$\frac{1}{8}$ m	-1	+5	+9	+10
.1168 m .....	$\frac{1}{16}$ m	+11	+22	+31	+41
.05906 m .....	$\frac{1}{32}$ m	+26	+47	+64	+87

It is quite evident from the above table that the absorption is dependent on certain main factors, viz.: (1) time of action of the solution, (2) concentration of the solution, (3) nature of the substance in solution. It will be observed that the effects during the first hour are much more marked, relatively speaking, than during the other intervals. As will be shown later, the effects are not purely those due to the differences in osmotic pressure on both sides of the sarcolemma, but are, rather, especially in the case of salts, a mixture of osmotic and specific molecular or ionic effects, together with effects due to metabolic changes. It may, however, be said here, that in the earlier intervals osmotic pressure effects are much the more prominent.

The effect of concentration of the solution may also be noted in the above table. It appears to be a general rule that hyper-tonic solutions bring about a negative absorption during the first intervals, while the iso- and hypo-tonic solutions cause a positive absorption. This negative absorption of the first few hours may or may not change in the later intervals to a positive one, according to the nature of the substance in solution.

Along with these factors which influence the action of solutions in phenomena of

absorption, must be mentioned certain conditions peculiar to the muscle which play a minor rôle in the phenomena: (1) difference in size (surface) of the muscles used may cause some variation in the average of absorption from any solution, inasmuch as adsorption phenomena are dependent on extent of surface. We should therefore expect to get more marked changes in muscles having surfaces relatively large as compared with the total mass of the muscle; (2) previous activity of the muscle may also vitiate the results, as in the active muscle certain metabolic products are formed (doubtless by enzymic activity), which may increase the osmotic pressure of the muscle-plasma; (3) a third variation is sure to creep in, owing to seasonal differences in the constitution of the frog's blood and tissues. This seasonal variation is shown in the following data of absorption from solutions isosmotic with  $\frac{1}{8}$ m NaCl:

TABLE III

Season	Substance	Concentration	ABSORPTION			
			1 h.	3 h.	6 h.	24 h.
Summer (Sept.)	CaCl <sub>2</sub>	$\frac{1}{8}$ m	- 5	-10	-22	-19
Winter (Mar.)..	CaCl <sub>2</sub>	$\frac{1}{8}$ m	+12	+18	+20	-10
Summer .....	NaCl	$\frac{1}{8}$ m	+ 0	+ 1	+ 2	+ 7
Winter .....	NaCl	$\frac{1}{8}$ m	+ 4	+ 4	+ 5	+ 6
Summer .....	KCl	$\frac{1}{8}$ m	+ 4	+12	+23	+39
Winter .....	KCl	$\frac{1}{8}$ m	+ 4	+ 7	+16	+55

In considering the results obtained in the absorption from cane-sugar solutions, we must take heed, therefore, of the concentration of the solution as well as its time of action. The chief question at issue, however, is whether we are dealing with a process explicable by the laws of osmotic pressure, or whether this process is due to some other physical or chemical change. From the above table of absorption from cane-sugar solutions, it will be noted that a muscle immersed in a hypertonic solution loses 53 per cent. of its weight in twenty-four hours, while in an isosmotic solution it gains 10 per cent., and in a hypotonic solution it gains 87 per cent. This cycle of absorption is exactly that which we should expect providing osmotic pressure effects were prominent. If a solution of salts be separated from a sugar solution by a membrane slowly permeable to the salts, quickly permeable to the water, and impermeable to the sugar, we should observe that water will pass from the salt solution into the sugar solution, providing the concentration of the sugar solution be greater than that of the salt solution. If the former be equal to or less than the latter, water will pass, to a less or great degree, from the sugar solution into the salt solution. This process is observed in phenomena of absorption from cane-sugar solutions.

As will be shown later, the sarcolemma of the muscle is slowly permeable to the ions of the salts within the muscle. Along with the passage of water from or into the muscle we will have, therefore, the passage of ions from the muscle into the sugar

solution. The ultimate result will be due, therefore, to a combination of osmotic pressure effects with those of diffusion.

c. *Absorption from Urea Solutions.*—The following table shows the absorption under influence of various concentrations of urea:

TABLE IV

Concentration	ISOSMOTIC NaCl Sol.	ABSORPTION			
		1 h.	3 h.	6 h.	24 h.
2.0659 m .....	$\frac{1}{2}$ m	— 5	— 5	— 4	+ 4
1.6745 m .....	m	— 5	— 4	— 4	+ 2
.8667 m .....	$\frac{1}{2}$ m	— 3	— 2	+ 1	+15
.4465 m' .....	$\frac{1}{2}$ m	+ 5	+14	+23	+42
.2286 m .....	$\frac{1}{2}$ m	+11	+29	+45	+72
.1168 m .....	$\frac{1}{4}$ m	+27	+46	+65	+66
.05906 m .....	$\frac{1}{8}$ m	+29	+42	+54	+53

It will be seen above that a muscle, immersed in a solution of urea isosmotic with  $\frac{1}{2}$  m NaCl, gains in one hour 11 per cent., while in twenty-four hours the gain is 72 per cent. What do these facts denote? If the sarcolemma be impermeable to urea, as it was to cane-sugar, then, of course, we should have the phenomena controlled by laws of osmotic pressure. If it be permeable to urea, as well as to water, we should have, according to laws of diffusion, urea passing from point of higher to that of lower concentration, or, in other words, into the muscle. An effort would be made to adjust the equilibrium on both sides of this permeable membrane. Along with this partial adjustment of urea, must go the adjustment of the concentration of various ions on each side of the sarcolemma. A current of diffusion is thus set up, which passes in both directions through the sarcolemma. The result of all this would, therefore, be an increase in weight on the part of the muscle.

It is clear that osmotic or ionic effects cannot explain the large increase in weight under influence of urea solution. The progress of absorption from urea is, markedly, similar to that noted when water is used, although the absolute absorption in the latter case is more marked at each interval except the final one. As the results seem to show, urea easily penetrates the sarcolemma. Osmotic pressure effects cannot, therefore, be considered of the first importance. Just why such a marked increase in absorption occurs during the later intervals does not seem clear to the writer at present. It may be that certain decided changes, such as the breaking down of urea into  $\text{NH}_4$  compounds or the formation of amido compounds, may result in an increased osmotic pressure and a resulting increase in absorption. The action of these nonelectrolytes upon phenomena of absorption is markedly different from that observed in other phenomena. Loeb, Lingle, Moore, Kahlenberg, True, and others have observed that, in general, nonelectrolytes have little or no effect on the phenomena under observation. In these experiments it is quite evident that the effect of the non-electrolytes is very marked.

## VI. EFFECTS OF ELECTROLYTES

*a. Halogen Salts.*—In selecting the salts to be used in this investigation, attention was paid to the results previously obtained by Loeb. He had shown that the effect of the halogens was, practically, the same for any given metal, although the absorption increased slightly in the order: chlorides, bromides, iodides, and fluorides. It seemed unnecessary, therefore, to use in these experiments solutions of all the haloids. As the type of halogen salt, sodium chloride, was selected, and the solutions of other salts were made equimolar with the NaCl solutions. The concentrations of these solutions were, as previously stated, graded from  $\frac{1}{2}$  m to  $\frac{1}{32}$  m, in order to permit of study of variations due to differences of osmotic pressure of same salt.

At the outset of the work on the influence of electrolytes, the importance of the third factor, mentioned above as influencing the process of absorption, viz., the nature of the substance in solution, was observed. Van Bemmelen, it will be remembered, found that the absorption by  $\text{SiO}_2$  depended, to a great extent, on the solution to be absorbed. In our work the nature of the cation, as well as of the anion of the salt used, influenced the absorption.

These facts are seen from the following table:

TABLE V

Salt	Concentration	ABSORPTION			
		1 h.	3 h.	6 h.	24 h.
NaCl.....	$\frac{1}{2}$ m	+0	+ 1	+ 2	+ 7
KCl.....	$\frac{1}{2}$ m	+4	+12	+23	+39
$\text{NH}_4\text{Cl}$ .....	$\frac{1}{2}$ m	+1	+ 3	+10	+29
$\text{CaCl}_2$ .....	$\frac{1}{10}$ m	-4	-21	-24	-18
$\text{MgCl}_2$ .....	$\frac{1}{10}$ m	+3	+ 7	+ 9	+18
$\text{Na}_2\text{SO}_4$ .....	$\frac{1}{10}$ m	+2	+ 6	+ 8	+ 6
$\text{K}_2\text{SO}_4$ .....	$\frac{1}{10}$ m	+1	+ 3	+ 6	+ 7

It is noted from above that in a solution of NaCl the muscle gains only 7 per cent., in isosmotic KCl it gains 39 per cent., while in  $\text{CaCl}_2$  solution it loses 18 per cent. The influence of the cation is seen when one compares the absorption under  $\text{Na}_2\text{SO}_4$  and  $\text{K}_2\text{SO}_4$  with that under NaCl and KCl. While the direct absorption under influence of the  $\text{SO}_4$  ion is not, in itself, so marked, there must be some strong anion effect to offset the marked cation effect noted under action of KCl.

From the data of above table we see that the halogens of the alkali and of the alkaline-earth group are divided into two classes, according to their power of hindering or facilitating absorption of liquid by animal tissues. Ca ions stand out, prominently, as the one inhibiting absorption, while Na shows a slight effect only, and K,  $\text{NH}_4$ , Mg,  $\text{SO}_4$ , all have a favoring effect.

The following table gives a complete list of the various halogens used, together with their concentration and time effects. The figures given under each concentration are the average figures of six experiments each:

TABLE VI

Salt	Concentration	ABSORPTION				Salt	Concentration	ABSORPTION			
		1 h.	3 h.	6 h.	24 h.			1 h.	3 h.	6 h.	24 h.
NaCl.....	$\frac{1}{2}$ m	-23	-20	-10	+11	CaCl <sub>2</sub> .....	$\frac{1}{2}$ m	-23	-22	-18	-18
NaCl.....	m	-17	-16	-7	+9	CaCl <sub>2</sub> .....	m	-21	-19	-15	-5
NaCl.....	$\frac{1}{2}$ m	-13	-12	-5	+6	CaCl <sub>2</sub> .....	$\frac{1}{2}$ m	-18	-14	-8	+2
NaCl.....	$\frac{1}{4}$ m	-7	-7	-7	+3	CaCl <sub>2</sub> .....	$\frac{1}{4}$ m	-16	-14	-12	-1
NaCl.....	$\frac{1}{8}$ m	+0	+1	+2	+7	CaCl <sub>2</sub> .....	$\frac{1}{8}$ m	-21	-18	-13	-2
NaCl.....	$\frac{1}{16}$ m	+13	+22	+26	+41	CaCl <sub>2</sub> .....	$\frac{1}{16}$ m	-18	-15	-9	-4
NaCl.....	$\frac{1}{32}$ m	+25	+42	+53	+75	CaCl <sub>2</sub> .....	$\frac{1}{32}$ m	-15	-19	-21	-11
NaF.....	$\frac{1}{2}$ m	-9	-13	-7	+13	CaCl <sub>2</sub> .....	$\frac{1}{2}$ m	-13	-17	-25	-22
NaF.....	$\frac{1}{4}$ m	+1	+4	+7	+22	CaCl <sub>2</sub> .....	$\frac{1}{4}$ m	-12	-20	-26	-20
KCl.....	$\frac{1}{2}$ m	-18	-10	-2	+21	CaCl <sub>2</sub> .....	$\frac{1}{8}$ m	-7	-26	-24	-22
KCl.....	m	-21	-15	-6	+17	CaCl <sub>2</sub> (Sept)	$\frac{1}{16}$ m	-4	-21	-24	-18
KCl.....	$\frac{1}{2}$ m	-15	-11	-3	+17	CaCl <sub>2</sub> (Mar.)	$\frac{1}{32}$ m	+12	+18	+20	-10
KCl.....	$\frac{1}{4}$ m	-5	-5	+2	+19	CaCl <sub>2</sub> .....	$\frac{1}{64}$ m	-1	-17	-23	-14
KCl.....	$\frac{1}{8}$ m	+4	+12	+23	+39	CaCl <sub>2</sub> .....	$\frac{1}{128}$ m	-4	-18	-21	-15
KCl.....	$\frac{1}{16}$ m	+20	+40	+54	+56	CaCl <sub>2</sub> .....	$\frac{1}{256}$ m	-7	-22	-26	-17
KCl.....	$\frac{1}{32}$ m	+25	+50	+63	+70	MgCl <sub>2</sub> .....	$\frac{1}{2}$ m	-18	-18	-11	+24
NH <sub>4</sub> Cl.....	$\frac{1}{2}$ m	-12	-5	+10	+20	MgCl <sub>2</sub> .....	m	-15	-12	-2	+30
NH <sub>4</sub> Cl.....	m	-12	-7	+4	+21	MgCl <sub>2</sub> .....	$\frac{1}{2}$ m	-10	-8	-4	+30
NH <sub>4</sub> Cl.....	$\frac{1}{2}$ m	-11	-12	-6	+10	MgCl <sub>2</sub> .....	$\frac{1}{4}$ m	-5	-1	+5	+32
NH <sub>4</sub> Cl.....	$\frac{1}{4}$ m	-6	-8	-6	+20	MgCl <sub>2</sub> .....	$\frac{1}{8}$ m	-3	-3	-1	+3
NH <sub>4</sub> Cl.....	$\frac{1}{8}$ m	+1	+3	+10	+29	MgCl <sub>2</sub> (Mar.)	$\frac{1}{16}$ m	+10	+21	+27	+36
NH <sub>4</sub> Cl.....	$\frac{1}{16}$ m	+14	+30	+46	+67	BaCl <sub>2</sub> (Mar.)	$\frac{1}{32}$ m	+12	+22	+30	+33
NH <sub>4</sub> Cl.....	$\frac{1}{32}$ m	+27	+49	+66	+54						

b. *Nitrates*.—Concerning the action of nitrates, it is to be said that the NO<sub>3</sub> radical does not seem to differ, in its action, from the Cl ion, inasmuch as the absorption by a muscle immersed in a nitrate solution is of the same order of magnitude as the absorption by a muscle in the corresponding chloride solution. The conclusion is evident, therefore, that in the experiments with the salts of monobasic acids, the cation is the influencing factor, while the anion acts indifferently. Isosmotic solutions of the nitrates were used in all cases as the general effect of hyper-, iso-, and hypo-tonic solutions were identical with those noted under effect of chlorides.

TABLE VII

Salt	Concentration	ABSORPTION			
		1 h.	3 h.	6 h.	24 h.
NaNO <sub>3</sub> .....	.12568 m	+0	+3	+5	+7
KNO <sub>3</sub> .....	.1258 m	+5	+12	+21	+50
NH <sub>4</sub> NO <sub>3</sub> ...	$\frac{1}{2}$ m	+2	+5	+14	+34
Ca(NO <sub>3</sub> ) <sub>2</sub> ..	$\frac{1}{16}$ m	-10	-18	-24	-19

c. *Sulphates*.—In the experiments first performed, the concentrations of the sulphate solutions were made equimolar with the chloride solutions. Here the same general effects were noted as in the case of the monobasic salts, viz., marked loss of fluid when muscle was placed in hypertonic solutions, while in hypotonic solutions of the same salt a marked absorption was observed.

The effect of the cation, in combination with the anion  $\text{SO}_4$ , was shown by the earlier experiments to be very slight.

TABLE VIII

Salt	Concentration	ABSORPTION			
		1 h.	3 h.	6 h.	24 h.
$\text{Na}_2\text{SO}_4$ .....	$\frac{1}{2}$ m	-4	-6	-7	-10
$\text{K}_2\text{SO}_4$ .....	$\frac{1}{2}$ m	-1	-2	-2	-7
$(\text{NH}_4)_2\text{SO}_4$ ....	$\frac{1}{2}$ m	-3	-4	-4	-10
$\text{Li}_2\text{SO}_4$ .....	$\frac{1}{2}$ m	-4	-6	-7	-10

Inasmuch as these effects of equimolar solutions were so very nearly identical, the further experiments on effects of isosmotic solutions of the various sulphates were assumed to be unnecessary. The effect of isosmotic sulphate of sodium was, therefore, taken as a general effect of such solutions of sulphates. We notice from the above table, as well as the one given below, that the action of the sulphates seems to be dependent on the effect of the anion, while the effect of the chlorides and nitrates depends on the action of the cation.

Below is given a complete table of sulphates used together with their concentration and time effects:

TABLE IX

Salt	Concentration	ABSORPTION				Salt	Concentration	ABSORPTION			
		1 h.	3 h.	6 h.	24 h.			1 h.	3 h.	6 h.	24 h.
$\text{Na}_2\text{SO}_4$ .....	$\frac{1}{2}$ m	-16	-19	-15	-4	$\text{K}_2\text{SO}_4$ .....	$\frac{1}{2}$ m	-8	-11	-13	-12
$\text{Na}_2\text{SO}_4$ .....	$\frac{1}{2}$ m	-16	-21	-18	-11	$\text{K}_2\text{SO}_4$ .....	$\frac{1}{2}$ m	-1	-2	-2	-7
$\text{Na}_2\text{SO}_4$ .....	$\frac{1}{2}$ m	-16	-23	-20	-6	$\text{K}_2\text{SO}_4$ .....	$\frac{1}{2}$ m	+8	+16	+24	+28
$\text{Na}_2\text{SO}_4$ .....	$\frac{1}{2}$ m	-10	-12	-19	-17	$\text{K}_2\text{SO}_4$ .....	$\frac{1}{2}$ m	+22	+41	+65	+67
$\text{Na}_2\text{SO}_4$ .....	$\frac{1}{2}$ m	-8	-12	-18	-9	$(\text{NH}_4)_2\text{SO}_4$ ...	$\frac{1}{2}$ m	....	....	....	-12
$\text{Na}_2\text{SO}_4$ .....	$\frac{1}{2}$ m	-4	-6	-7	-10	$(\text{NH}_4)_2\text{SO}_4$ ...	$\frac{1}{2}$ m	....	....	....	-11
$\text{Na}_2\text{SO}_4$ .....	$\frac{1}{2}$ m	+2	+6	+8	+4	$(\text{NH}_4)_2\text{SO}_4$ ...	$\frac{1}{2}$ m	-3	-4	-4	-10
$\text{Na}_2\text{SO}_4$ .....	$\frac{1}{2}$ m	+1	+8	+10	+13	$(\text{NH}_4)_2\text{SO}_4$ ...	$\frac{1}{2}$ m	+3	+7	+7	+3
$\text{Na}_2\text{SO}_4$ .....	$\frac{1}{2}$ m	+10	+21	+28	+33	$(\text{NH}_4)_2\text{SO}_4$ ...	$\frac{1}{2}$ m	....	....	....	+20
$\text{Na}_2\text{SO}_4$ .....	$\frac{1}{2}$ m	+12	+26	+35	+41	$(\text{NH}_4)_2\text{SO}_4$ ...	$\frac{1}{2}$ m	....	....	....	+44
$\text{Na}_2\text{SO}_4$ .....	$\frac{1}{2}$ m	+25	+45	+55	+66	$\text{Li}_2\text{SO}_4$ .....	$\frac{1}{2}$ m	-4	-6	-7	-10
$\text{Na}_2\text{SO}_4$ .....	$\frac{1}{2}$ m	+30	+50	+57	+48	$\text{MgSO}_4$ .....	$\frac{1}{2}$ m	+10	+19	+26	+35
$\text{K}_2\text{SO}_4$ .....	$\frac{1}{2}$ m	-13	-23	-26	-17						

*d. Oxalates and Carbonates.*—The absorption noted, when the influence of oxalate and carbonate solutions is studied, is as follows:

TABLE X

Salt	Concentration	ABSORPTION			
		1 h.	3 h.	6 h.	24 h.
$\text{Na}_2\text{C}_2\text{O}_4$ .....	$\frac{1}{2}$ m	-10	-12	- 6	+20
$\text{Na}_2\text{C}_2\text{O}_4$ .....	$\frac{1}{4}$ m	- 4	- 6	- 7	+ 0
$\text{Na}_2\text{C}_2\text{O}_4$ .....	$\frac{1}{8}$ m	+ 8	+12	+21	+30
$\text{Na}_2\text{C}_2\text{O}_4$ .....	$\frac{1}{16}$ m	+12	+23	+31	+42
$\text{Na}_2\text{CO}_3$ .....	$\frac{1}{2}$ m	0	+10	+21	+45
$\text{Na}_2\text{CO}_3$ .....	$\frac{1}{4}$ m	+ 2	+15	+27	+53
$\text{Na}_2\text{CO}_3$ .....	$\frac{1}{8}$ m	+12	+20	+28	+39
$\text{Na}_2\text{CO}_3$ .....	$\frac{1}{16}$ m	+16	+30	+33	+45

From the above tables we see that the effect of the anions  $\text{SO}_4$ ,  $\text{C}_2\text{O}_4$ ,  $\text{CO}_3$ , is much different from that of the Cl anion. In equimolar solutions there seems to be a close analogy between the effects of the dibasic group and those of calcium salts of monobasic acids. These effects were to be expected if the idea of Wallace and Cushny, concerning the precipitation of calcium by saline cathartics, be correct. If, however, we compare isosmotic solutions, the apparent retarding effect of the anion is replaced by a favoring effect. Thus it will be observed that a muscle gains, in  $\frac{1}{10}$  m  $\text{Na}_2\text{SO}_4$ , 4 per cent in 24 hours, while in  $\frac{1}{10}$  m  $\text{CaCl}_2$ , it loses 20 per cent.

An apparent variation from the general effect of dibasic anions is noted in the case of  $\text{Na}_2\text{CO}_3$  solutions. Here we notice that a positive absorption begins, at once, and increases much more rapidly than even that due to  $\frac{1}{2}$  m KCl solution. It will be remembered that Loeb found that the addition of a small number of OH ions greatly facilitated the absorption. In  $\text{Na}_2\text{CO}_3$  solution we have quite a marked hydrolysis, giving us free OH ions, upon which depends the alkaline reaction of the solution. We are, therefore, justified in assuming that the marked positive effect, noted in case of  $\text{Na}_2\text{CO}_3$ , is due to the free OH ions present in the solution.

*e. Citrates.*—The results following the use of citrate solutions were, practically, the same as those observed with dibasic salts. Here, again, we notice that isosmotic solution ( $\frac{1}{12}$  m) of sodium citrate has a slightly favoring effect, instead of an inhibiting one. Although the absorption under the influence of dibasic and tribasic salts is not at all great, yet the variation, noted in the effects of Na and K salts of monobasic acids, is lost, when we observe the results of absorption under Na and K salts of polybasic acids. The anion must, therefore, exert some influence on the absorption, either by neutralizing the effect of the cation as in the case of  $\text{K}_2\text{SO}_4$  or, perhaps, by changing the state of the muscle plasma by the precipitation of calcium.



TABLE XI

Salt	Concentration	ABSORPTION			
		1 h.	3 h.	6 h.	24 h.
$\text{Na}_2\text{C}_6\text{H}_5\text{O}_7 \dots$	$\frac{1}{4}$ m	- 6	- 9	-10	- 9
$\text{Na}_2\text{C}_6\text{H}_5\text{O}_7 \dots$	$\frac{1}{8}$ m	+ 4	+ 4	+ 3	+ 3
$\text{Na}_2\text{C}_6\text{H}_5\text{O}_7 \dots$	$\frac{1}{16}$ m	+11	+20	+28	+39

## VII. EFFECTS OF TEMPERATURE ON ABSORPTION PHENOMENA

Van Bemmelen found, in his work on inorganic colloids, that the power of absorption decreased with the temperature. This, he explained, was due to a change in the structure of the colloid, which change resulted in the transformation of the colloid into a much denser physical state. He says that every change in structure of a colloid causes a change in the absorption power of a colloid, if this structure be non-reversible when brought into contact with a solution. All such modifications cause (1) a thickening or drawing together of the walls of the colloid, (2) a narrowing of spaces in the substance of the colloid, (3) a change of capillary force, and (4) a consequent change of the absorption coefficient of the colloid.

As we are dealing with a non-reversible gel, the protoplasm, we should expect to find that any modification, brought about by temperature, in the muscle, would show the decrease of absorptive power spoken of above.

Our first experiments bearing on this point were made with temperatures easily found in the laboratory. The muscles were placed in the solutions and allowed to remain at the same temperature for 24 hours, at end of which time they were weighed and their absorption calculated.

The results of these first experiments showed that, in the range of temperatures found in the laboratory, absorption effects were practically the same for the same salt and same concentration of this salt. We, therefore, arranged a series of experiments in which the muscle was put into rigor by being heated to 50–52°C in a solution of  $\frac{1}{8}$  m NaCl for 10 minutes. After being thus treated the muscle is dried, weighed, and placed in certain solutions for 24 hours. Control experiments were made with muscle not in rigor. The striking effects may be observed from the following table.

From above data it is quite evident that some change has occurred in the muscle which lessens the power of absorption. The result is most striking in cases of  $\frac{1}{8}$  m KCl and  $\frac{1}{8}$  m  $\text{CaCl}_2$ . We see here that a muscle in rigor absorbs only a very slight amount of fluid if placed in a KCl solution while in a  $\text{CaCl}_2$  solution a much greater absorption or, rather, a smaller negative absorption is observed. The conclusions are quite evident that some structural change has occurred in the muscle which modifies the absorptive power of the muscle. Comparing this result with those of van Bemmelen we are led to believe that the structure of the muscular protoplasm is much like that of the gel of  $\text{SiO}_2$ , viz., a web-like structure of colloid, holding, in its micellary and

TABLE XII

Salt	Concentration	State of Muscle	Absorption in 24 Hours
KCl.....	$\frac{1}{2}$ m	Rigor	+ 3
KCl.....	$\frac{1}{2}$ m	Normal	+45
NaCl.....	$\frac{1}{2}$ m	Rigor	+ 8
NaCl.....	$\frac{1}{2}$ m	Normal	+ 8
NaCl.....	$\frac{1}{2}$ m	Rigor	+11
NaCl.....	$\frac{1}{2}$ m	Normal	+27
NaCl.....	m	Rigor	+ 9
NaCl.....	m	Normal	+20
CaCl <sub>2</sub> .....	$\frac{1}{2}$ m	Rigor	- 3
CaCl <sub>2</sub> .....	$\frac{1}{2}$ m	Normal	-22

interstitial spaces, fluid by capillary attraction, adsorption, and absorption. The recent experiments of Greeley confirm this idea of change of structure under influence of temperature and consequent change in absorptive power of muscle.

### VIII. THEORETICAL CONSIDERATIONS

#### I. PERMEABILITY OF SARCOLEMMMA

In discussing the theoretical conclusions to be drawn from the experiments detailed in this paper, the question to be first settled is whether the protoplasm of the muscle is permeable and if so in which direction or directions and to what substances.

It will be remembered that in former papers Lingle, Loeb, and Miss Moore called attention to the fact that the rhythmical contractions of strips of turtle's ventricle, of skeletal muscle, and of the lymph hearts of frogs, were under the influence of certain ions. They further showed that electrolytes caused an effect while non-electrolytes caused none. Loeb's explanation of the phenomenon noted in skeletal muscle was that a certain physiological balance existed between the inorganic cations of the muscle plasma. If this balance be disturbed certain phenomena, among them rhythmical contractions of the muscle, are observed. He shows, as do also the other authors noted above, that the contractions are due to the excess of Na ions beyond that physiological relation existing between Na and Ca. If a muscle be immersed in a sodium chloride solution Na ions, says Loeb, will penetrate the muscle and will gradually replace the Ca, K, and other cations present. In about an hour rhythmical contractions will begin and will last for some time. If, instead of an NaCl solution, a Na<sub>2</sub>SO<sub>4</sub> solution be used, these contractions appear at once, because, as he says, the physiological relation has been disturbed both by entrance of Na ions and by the precipitation of the Ca ions by SO<sub>4</sub> radical. This latter result is more striking if an alkaline oxalate or citrate solution be used instead of a sulphate solution. It was further shown by these authors that Ca, K, Mg, Ba, etc., had exactly the opposite effect upon rhythmical contractility, viz., an inhibiting action. It seems quite evident, from these

results, that we must admit the permeability of the muscle membrane to the anion as well as to the cation of the salts investigated. However, it must be said that the effect of the anion seems to have no greater significance than that those anions precipitating Ca act much more readily than the others by disturbing the physiological balance more quickly than do the anions Cl, Br, I, NO<sub>3</sub>, etc.

Concerning the permeability of the sarcolemma to non-electrolytes such as sugar, urea, water, etc., we have little literature to draw from. The above mentioned authors show that rhythmical contractions do not arise in solutions of non-electrolytes. However, from the absorption phenomena noted in case of cane sugar and urea we notice that the sarcolemma is permeable to urea and not to cane-sugar solutions.

Having shown the permeability inward of the sarcolemma for the various substances investigated, our next question seems to be, Is the sarcolemma permeable outward to these same substances? It is a well-known fact that the red blood-corpuscles of most animals contain more potassium than sodium while the serum of the same animal contains these elements in the reverse relation. This same thing is noticed in case of composition of muscle plasma. These facts seem to show that the corpuscles and muscles are impermeable to the Na and K ions, else we should have an equilibrium established between these ions on both sides of the membranes in question. The work, previously mentioned, on red blood-corpuscles seems to show that the corpuscular membrane is impermeable to K, Na, Ca, sugar, etc., while it is permeable for NH<sub>4</sub>Cl, urea, etc. Our work on muscle shows that the sarcolemma is permeable for Na, K, Ca, NH<sub>4</sub>, urea, etc., while it is impermeable to sugar. How account for these variations? As we have seen above, differences in osmotic pressure were given as the cause of the phenomena noted in red blood-corpuscles, while, in our work, these differences do not explain the effects of the various substances used. The answer to these questions might be that in case of blood corpuscles we are dealing with a membrane which is permeable in neither direction to certain ions, while in muscle we have to do with a membrane permeable in both directions although the rate of diffusion is different for each ion and is different in the two directions. That this latter statement is correct may be readily seen from the following: The permeability inward of the sarcolemma is evident from the experiments on rhythmical contractions of muscle. If a muscle be placed in a solution of CaCl<sub>2</sub>, as we have seen, Ca and Cl ions will enter the muscle and will inhibit or restore rhythmical contractions of a muscle according to the state of a muscle previous to immersion in the solution. It can be definitely shown that K and, possibly, Na ions have penetrated outward from the muscle by a simple qualitative chemical test of the solution in which the muscle has been immersed. By addition to this solution of hydrochlorplatinic acid, evaporation of the mixture nearly to dryness, and then the addition of alcohol, we obtain the characteristic reddish-yellow octohedra of K<sub>2</sub>PtCl<sub>6</sub>. It is to be remembered here that the detection of Na in presence of K is not possible by this test as the Na<sub>2</sub>PtCl<sub>6</sub> is soluble in the alcohol used. We can, therefore, state definitely that K ions have penetrated outward and that in all probability the Na ions have also done the same. Do Ca ions also

penetrate outward? If a muscle be placed in a solution of  $\text{Na}_2\text{SO}_4$  rhythmical contractions are, as mentioned above, observed at once. If the muscle be allowed to remain in these solutions 12 hours a slight opalescence is observed which becomes more marked at the end of 24 hours and may even pass into a distinct precipitate. On examination of this precipitate by ordinary qualitative methods, Ca was, unmistakably, shown by its characteristic oxalate. There can be no doubt, therefore, of the permeability outward of the sarcolemma in case of Ca ions. This latter reaction must not be confused with another which is observed in practically all of the salt solutions used, although it was more marked in case of the solutions whose anion precipitated Ca. The reaction in question is as follows: If a muscle be placed in certain salt solutions, preferably the sulphate or oxalate of alkali metals, there will be observed in 18 hours a decided opalescence. This turbidity is not all due to the precipitation of Ca salts as is seen from the experiment. The opalescent solution is heated to  $65-75^\circ\text{C}$  for a few minutes, when a distinct coagulum appears which resembles that of white of egg. The solution has a strong proteid odor and shows a distinct froth on its surface. Tested with  $\text{CuSO}_4$  and  $\text{KOH}$  a distinct violet coloration is noted. This seemed to point to the presence of proteid matter in the solution. In order to confirm this assumption it seemed necessary only to test for carbon and nitrogen. By the ordinary organic methods of examination both these elements were detected, showing conclusively the presence of proteid. Here we have, evidently, to do with the solvent action of the salt solution upon the proteid matter of the muscle. We are, however, not in a position to give the origin of this proteid. Two possibilities present themselves for consideration. In the first place we may have a simple solvent action of the salt solution upon the surface of the muscle. Secondly, we might conceive of some combination of the proteid, within the sarcolemma, with the salt, which combination is diffusible. The first possibility seems much more plausible but cannot be absolutely proven by these experiments.

The sarcolemma of the muscle is, therefore, permeable to the ions under investigation. However, it is readily seen from the previous points that the rate of movement of the ions is different in each direction. Whereas, for instance, the inward penetrability of the Na ion may be definitely shown within a few minutes, the outward penetrability may not be detected for several hours. Moreover, we know that the rate of diffusion of Ca ion is much slower than that of the Na and approximately the same as the K ion because, according to Graham's law of diffusion of gases, we should expect the rate of diffusion to be inversely proportional to the square root of the density of a solution. Reid has shown that the rate of osmosis through the skin of a frog is different in each direction. We are, hence, justified in our assumption here, that the sarcolemma of the muscle is permeable in both directions to certain ions and that the rate of diffusion is different in each direction being much faster inward than outward. In this passage of ions into and out from a muscle we have many more ions entering the muscle than leaving during the same interval of time owing to the factors (1) of rate of migration of ion and (2) direction of migration. Just how far these assump-

tions affect the permeability of the muscle for non-ionized substances, such as cane sugar and urea, we are not in a position to state, as no direct experiments were made bearing on the point of outward penetrability of urea. Cane sugar, it will be remembered, does not seem to penetrate the sarcolemma at all and therefore obeys perfectly the laws of osmotic pressure.

## II. OSMOTIC PRESSURE EFFECTS

a. At the beginning of this section it may be asserted that differences in osmotic pressure, on both sides of the sarcolemma, do not account for the results of absorption of fluid by muscle. Hamburger's work on the red-blood corpuscle apparently proved that such was the case in phenomena noted in his experiments. He, however, neglected the consideration of partial osmotic pressure as well as the question of permeability of corpuscular membrane to certain ions, in which latter case osmotic pressure could play no rôle whatever. From our own experiments on blood-corpuscles we find, by use of Hedin's hæmatokrit method, that the corpuscles do, apparently, obey osmotic laws if solutions of K, Na, Ca, etc., salts be used. Yet we cannot assert from these experiments that these laws are the only ones governing the phenomena noted, inasmuch as our work on the muscle shows us, conclusively, that osmotic effects are accountable for slight changes only.

b. Generally speaking, a muscle immersed for a short interval in a hypertonic solution of an electrolyte or non-electrolyte will lose in weight; if placed in an isotonic solution it may or may not gain, while in a hypotonic solution of the same substance it regularly increases in weight. Miss Cooke has found, it will be recalled, that in a solution of  $\frac{1}{2}$  m NaCl (5.1087 atmospheres pressure) a muscle neither gains nor loses in weight in eighteen hours. The assumption was very natural that this NaCl solution was isosmotic with the muscle plasma, as Loeb's previous work showed that the effects of acids and alkalis were proportional to the number of free H and OH ions present in the solutions. This assumption is, however, open to several serious objections. In the first place it has been shown by Pfeffer, Linebarger, Picton and Linder, Sabanejew, Tamman, and others, that colloids have, in solution, slight osmotic pressure values. In the second place, as we have shown above, Na, K, Ca,  $\text{SO}_4$ , Cl ions penetrate the sarcolemma in both directions though at a varying rate in each direction. We must, therefore, conclude that the laws of osmotic pressure do not explain the phenomena noted in these absorption experiments. The markedly different effects of isosmotic solutions of various chlorides show us that the ionic effects far exceed the osmotic effect noted in these experiments.

c. If the question of interchange of ions or molecules through animal membranes be considered, we find that the relative semi-permeability to ions must determine whether or not osmotic pressure laws are valid in such experiments. Our experiments show clearly that we are dealing in this phenomenon of absorption of fluid by muscle with a combination of ionic effects on the one hand with those of osmotic pressure and hydro-diffusion on the other. The laws of diffusion state that substances, obeying

these laws, pass from the point of higher to that of lower concentration of these substances and, of course, show a greater velocity the higher the temperature. A free interchange of ions between the solution outside and that inside the sarcolemma is naturally prevented by the relative permeability of sarcolemma as well as the varying velocity of the ions in the two directions. As the rate of diffusion is dependent on the density of the solution, the amount of fluid passing into a muscle from a hypertonic solution would be much less than that entering from a hypotonic solution. Such is the case as observed in our experiments, yet it is more than probable that osmotic effects play a much greater rôle in these stronger and weaker solutions than in isosmotic solutions where the ionic effects are more marked.

Just how far the sarcolemma influences the process of diffusion cannot be stated, inasmuch as our knowledge of the structure of this membrane is meager. The views of Klein and van Beneden concerning the reticular structure of protoplasm are opposed by those of Bütschli, who advances the theory of "foam-structure" of protoplasm. Recent work by Hardy upon organic colloids and van Bemmelen upon inorganic colloids show that the structures with which they were dealing in their experiments were web-like structures in the interstitial spaces of which fluid is held by capillary attraction, adsorption, and absorption.

Brücke has advanced a theory of "pore diffusion" to explain the effect of an animal membrane upon diffusion. He assumes capillary spaces in the membrane, which spaces hold a layer of liquid by capillary attraction. If the space be very small, then, of course, the membrane becomes relatively semi-permeable. Fick, in addition to this idea of pore diffusion, assumes that this process occurs by a diffusion through the molecular aggregates making up the membrane. This latter process is, of course, dependent on laws of adsorption.

We are, therefore, not in a position to say exactly what influence the sarcolemma exerts upon the absorption noted in our experiments, but we can state that a much more complicated process is involved here than would be the case if the sarcolemma were absolutely impermeable to the substances used.

d. The element of time plays an important rôle in the phenomena observed in our experiments. The effect during the first intervals is much more marked, relatively speaking, than during the later ones and is also, in hypertonic solutions, of a different phase. What do these variations denote? We might assume that the physiological condition of the sarcolemma and of the muscle as a whole has been markedly affected by the solution used. This assumption is borne out by Reid, who showed that the passage of fluid through the skin of the frog is intimately connected with the physiological condition of the tissue. He further showed that agents which tend to depress the vital activity diminish the osmosis in the normal direction, while those agents stimulating activity give rise to an increase in osmosis. It has also been shown by Loeb, Lingle, Moore, and Kahlenberg and True that certain salts act as definite protoplasmic poisons. Loeb pointed out that pure solutions of NaCl, KCl, CaCl<sub>2</sub> are poisonous as far as contractile power of muscle is concerned. Miss Moore, in her work on trout

and on the lymph-heart of frogs, showed that the solutions mentioned above have poisonous effects in these latter cases. We are, therefore, supported in the assumption that the time effects may be due to certain physiological changes in the muscular tissues resulting, possibly, in formation of various products of enzymic activity, which products would increase the osmotic pressure inside the muscle and therefore cause a later absorption of fluid.

### III. IONIC EFFECTS

(a) If a muscle be immersed in a  $\frac{1}{8}$ m NaCl solution for twenty-four hours it gains but slightly in weight. In a solution of  $\frac{1}{8}$ m KCl (isosmotic with  $\frac{1}{8}$ m NaCl) the gain is 40 per cent., while in a solution of  $\frac{1}{10}$ m CaCl the loss is 20 per cent. From the previous sections of this paper the variations in the effects of cations Na, K,  $\text{NH}_4$ , Ca, Mg, Ba, and anions  $\text{SO}_4$ ,  $\text{C}_2\text{O}_4$ ,  $\text{C}_6\text{H}_5\text{O}_7$ ,  $\text{CO}_3$ , Cl, and  $\text{NO}_3$  will be evident. It was shown above that osmotic pressure differences could not account for the divergence noted when isosmotic solutions of various salts were used. Loeb, in his work on absorption by muscle, advanced the following theory to explain the phenomenon noted at the beginning of this section. "Salts or electrolytes in general do not exist as such in the living tissues. In the muscle the various metal ions exist in combination with the proteid, in which combination they may be easily substituted, one for the other. In this substitution certain physical properties of colloid, especially their power of absorption of water and their state of matter, are changed." According to this "ion-proteid" theory, we should expect that, no matter what the outside solution might be, the ions of this solution would enter the muscle and substitute themselves for the ions present in the muscle. It is a well known chemical fact that we have combinations of metal ions with the radicals of the higher fatty acids forming a distinct class of compounds known as soaps. Potassium soaps absorb large amounts of water, in fact they constitute the class of soft-soaps, which have an almost fluid consistency. Sodium soaps absorb only a slight amount of water, while the calcium soaps are very insoluble and absorb none. The effects of ions upon absorption by muscle are to form "ion-proteids," which effects show a "remarkable parallelism with the influence of the same ions upon absorption of water by soaps." The effects of Ba and Mg ions are quite different from those of Ca ions. Chemically speaking, we might expect these former ions to behave like Ca ions, yet, physiologically considered, effects do not always follow chemical characteristics. Our first idea of the cause of this variation was that, inasmuch as  $\text{MgCl}_2$  is hydrolyzed in solution to  $\text{MgOHCl}$  and  $\text{HCl}$ , we might be dealing here with an action of the H ions of the  $\text{HCl}$ . This is not a very plausible conclusion, as the hydrolysis is so slight that such a marked result could hardly follow. This was further shown to be untenable when the action of  $\text{BaCl}_2$  was investigated. This salt is not at all hydrolyzed in solution and can therefore have no free H ions in its solution. As the effects of  $\text{BaCl}_2$  and  $\text{MgCl}_2$  are almost identical, we must assume that we are dealing with the same cause in each case. We therefore revert to the ion-proteid theory and conclude that Ba- and Mg-proteid compounds show much the same absorptive power as do the K-proteid combinations. That Ba and Mg soaps show this

same property of absorbing water may be doubted, as the soaps of the alkali-earth metals are all insoluble and show no phenomena of solid solution.

(b) The effect of the anion bound to the cation shows a marked variation in cases of Cl and  $\text{NO}_3$ , on the one hand, and the  $\text{SO}_4$  group on the other. From previous data we notice that the ions of Cl, Br, I, and  $\text{NO}_3$  influence, to a very slight extent, the absorption of fluid by a muscle, while those anions which precipitate calcium show a marked positive effect. In fact, in solutions of salts of the polybasic acids, used in our experiments, the anion was the all-powerful factor, while the cation plays an indifferent rôle. In isosmotic solutions of the sulphates (the metal group being indifferent) the absorption is between 4 and 10 per cent., agreeing, practically, with the absorption under influence of NaCl.

We must, therefore, assume that in this ion-proteid combination the anion, as well as the cation, is attached to the proteid molecule, but at a different point of the molecule. It thus appears that the group of salts, known pharmacologically as saline cathartics, is not, in reality, an inhibitor of absorption, but is rather an accelerator of this process. As the calcium salts seem to have a direct inhibiting effect on absorption by muscle, it may be possible that these cathartics may, by their precipitating action on the calcium contained in the muscle, exert an accelerating effect. This conclusion is hardly plausible, inasmuch as we have the same amount of absorption under the influence of NaCl.

(c) The assumption, therefore, of a combination of both anion and cation with the proteid of the muscle seems to be the most valid one. We must, however, admit that in this combination the effects of the anion are in some cases more prominent, while in others the action of the cation is the important factor.

That cations and anions both combine with the proteid has been directly shown by Pauli, Tangl and Bugarsky, Spiro, Atkinson, Stewart, and others. Our assumption of "ion-proteids" is therefore made valid by direct experimentation.

#### IV. INDEPENDENT ABSORPTION OF SALT AND WATER

The question naturally arises in this discussion, whether the increase in weight of a muscle immersed in a solution is due entirely to absorption of water or partly to water and partly to dissolved substance. Hofmeister has definitely shown in his work on absorption of solutions by gelatine disks, that an independent absorption of salt and of water takes place. That is, the solution is not absorbed as such, but water is absorbed up to a certain limit, depending on the concentration, while salt is taken up in quantities approximately proportional to concentration. Our work was not planned to show the dynamics of the absorption to any further extent than to prove the absorption of water on the one hand, or, on the other, the absorption of the salt along with the water.

It will be recalled that the sarcolemma is permeable to certain ions which have been shown to actually enter the muscle and form compounds with the proteid (colloids) of the muscle. We should, therefore, expect to find by gravimetric methods that salts had actually added to the original weight of the muscle. If we estimate the



dry weight of one gastrocnemius by placing it, as soon as it is removed from the body, in a dessicator containing sulphuric acid, for twenty-four hours, while the dry weight of the other gastrocnemius of the frog is estimated after it had been in a solution of  $\frac{1}{10}$  m Na<sub>2</sub>SO<sub>4</sub> for twenty-four hours, we should expect the dry weight of these two muscles to show the same slight variations noted in the original muscles, provided the increase in weight of the second muscle was due entirely to taking up of water. If an absorption of salt occurred in the second muscle, this absorption would of course be evident in an increase of the dry weight of the second over the first muscle. Such experiments were carried out with several muscles, the result being quite positive in all cases. From the following data the absorption of salt may be readily observed:

Wt. of orig. muscles in grams	Wt. after being in sol. 24 hrs.	Weight after drying 36 hrs.	Orig. difference in wt.	Final difference in wt.
2.313	3.42	.6347	.0189	.0315
2.2941		.6032		

The original difference in weight is markedly less, relatively speaking, than the final difference. Apparently, an absorption of .0126 grams of salt has taken place. It seems very plausible to conclude, from this composite experiment, that absorption of both water and salt has occurred but that a much larger absorption (98 per cent.) of water than of salt has taken place. If the solvent action of the solution upon the proteid, as well as the outward passage of Ca and other ions from the muscle, be remembered, it may be readily understood why the increase in dry weight is not more marked. It may be argued that the time of drying would necessarily influence the amount of water given off by the muscle when in the dessicator and that this slight increase was due to unequal time of evaporation. The experiment was continued for some time, daily observations being made, until the weights of the two groups of muscles remained constant for two consecutive days. We may in this case assume that the difference in weight, if any exists, is due to the taking up of salt by muscles immersed in the salt solution. The results follow:

Original weight	Wt. after being in sol. 24 hrs.	Dry wt. after 11 days	Orig. difference in wt.	Final difference in wt.
2.313	3.42	.4702	.0189	.0341
2.2941		.4361		

The difference has not disappeared on more complete drying. An interesting observation was brought out in these experiments. If we calculate, from figures given by Danilewsky, the amount of water in the above muscles, we find that the weight obtained by deducting the amount of water from the weight of the original muscle is larger than the weight obtained in our drying experiment. It is therefore clearly evident that Danilewsky's figures for per cent. of water in muscle are low or that in these experiments some abnormally watery muscles were used. In the following data these points may be noted, assuming, according to Danilewsky, 78.8 per cent. H<sub>2</sub>O in muscle:

Original weight	Amount of H <sub>2</sub> O 78.8 per cent.	Weight after deducting H <sub>2</sub> O	Weight after drying
2.313	1.823	.4900	.4702
2.2941	1.808	.4861	.4361

## IX. SUMMARY AND CONCLUSIONS

1. Muscles absorb fluid from hyper-, iso-, and hypo-tonic solutions of electrolytes and non-electrolytes.
2. The sarcolemma of the muscle is permeable to the ions Na, K, Ca, Mg, Ba, NH<sub>4</sub>, Li, Cl, Br, I, NO<sub>3</sub>, SO<sub>4</sub>, C<sub>2</sub>O<sub>4</sub>, CO<sub>3</sub>, C<sub>6</sub>H<sub>5</sub>O<sub>7</sub>, in both directions, but at a much slower rate outward than inward.
3. Osmotic effects account only for absorption from water and cane sugar solutions.
4. Specific ionic effects play an important role in absorption from solutions of electrolytes. The "ion-proteid" theory seems to fully explain all cases noted.
5. The effect of the anion is marked in solutions of the salts known as saline cathartics. Instead of an inhibiting effect on absorption, these anions favor this process to a certain degree.
6. Absorption from isosmotic solutions depend on (1) the nature of the substance in solution, (2) time of action of the solution, (3) relative permeability of sarcolemma, (4) physical state of the muscle, and (5) temperature at which absorption takes place.
7. K, NH<sub>4</sub>, Ba, and Mg cations seem to favor absorption, Na and Li are indifferent, while Ca cations show a marked inhibiting action on this process.
8. The anions Cl, Br, I, and NO<sub>3</sub> act indifferently toward the process of absorption, while anions of the SO<sub>4</sub> group show a positive effect.

In conclusion I wish to thank Professor Loeb for his many valuable suggestions both as regards methods of experimentation and interpretation of the results obtained.

## ADDENDUM

Since the manuscript of the above article was sent to press, two articles by E. Overton on allied subjects, have appeared: "Beiträge zur allgemeinen Muskel- und Nervenphysiologie," *Archiv f. d. ges. Physiol.*, Vol. XCII (1902), pp. 115 ff.; *ibid.*, pp. 346 ff.

These articles, although bringing out certain facts mentioned in above paper, show that Overton is not familiar with the entire literature of the subject.

It is hard to explain the known facts concerning nutrition, as well as those previously brought out by Loeb on rhythmic contraction of muscle under influence of certain ions, without granting the permeability of the muscle plasma for the ions in question. This fact is absolutely contradicted by Overton.

The second paper, dealing with the influence of Na ions on muscle contractility, is, on the whole, a confirmation of Loeb's work published in 1899 and 1900.

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**BLOOD-VESSELS IN THE LABYRINTH OF  
THE EAR**





## THE DISTRIBUTION OF BLOOD-VESSELS IN THE LABYRINTH OF THE EAR OF *SUS SCROFA DOMESTICUS*

GEORGE E. SHAMBAUGH

THE course of the blood-stream through the labyrinth of the ear has been a subject of considerable uncertainty and difference of opinion among anatomists. The reasons are obvious, for the complicated series of cavities that go to make up the labyrinth of the ear makes the study of its blood-supply by the ordinary method from sections very inaccurate unless laborious methods of reconstruction are used. This element of difficulty was successfully overcome, however, when the method was introduced of making celloidin casts of the labyrinth in which the circulation could be viewed in its entirety. But with this accomplished, and with such a complete picture of the blood-supply before one, the complicated network of vessels found in the labyrinth of the adult ear is in many places very difficult, if not quite impossible, to disentangle with any degree of accuracy.

It seemed desirable, therefore, in order to get a correct understanding of the circulation of the adult ear, to work out the angiogenesis of the labyrinth in the hope that in the younger embryo would be found a less complicated circulatory system which would serve as a guide in interpreting the more intricate system of vessels found in the adult ear. Since work of this kind undertaken on the human ear would necessarily be greatly hampered on account of the difficulty in securing sufficient material, it was decided to use the embryo pig, because an abundant supply of fresh material gave ample opportunity for experimenting with methods of injection, until satisfactory preparations could be obtained in sufficient numbers for study. It was thought that an accurate picture of the blood-supply obtained in this way for the labyrinth of the pig's ear would, perhaps, throw light upon some of the doubtful points in the circulation of the human ear. At any rate, such a knowledge of the circulation in the ear of the pig is desirable in itself. That the circulation in the labyrinth of the pig's ear would differ in some respects from that of the human ear is, of course, to be expected, but in general we should look for a more or less close resemblance between the two.

In injecting the vessels of the labyrinth in the embryo, many preparations were rendered useless, either because the pressure used was not sufficient to force the injecting fluid into the labyrinthine vessels, or because the pressure was too great for the delicate vessel walls, and the preparation was ruined by the escape of the injecting fluid into the surrounding tissue. It was found impossible always to gauge accurately the amount of pressure necessary to suit the different ages; hence it became necessary to inject a large number of embryos from which were selected those that proved satisfactory for study. Altogether over 500 embryos were injected, and

from this number scarcely more than 100 specimens proved suitable for study. Among these were preparations showing all degrees of injection, from the perfectly injected labyrinth having a complete capillary injection, to the semi-injected ones and those having only the larger trunks injected. In some there was a perfect injection of the arterial tree alone, up to the point where it breaks up into capillaries, and in several preparations there was a similar perfect injection of the venous tree. These latter preparations have been invaluable for determining the general scheme of the course of the arterial and venous streams through the labyrinth, which in the completely injected preparation is often quite obscured by the dense capillary injection about them. On the other hand, from this large number of injections, the completeness with which the capillaries were filled in a few of the preparations gave an opportunity for filling out with perfect accuracy this link between the arteries and veins.

The description that follows is confined to the results of my own work, reference being made only occasionally to the work of others. There is no attempt to cover the literature on the subject, which has been carefully done by Eichler.<sup>1</sup>

A noteworthy addition to that bibliography is the beautifully illustrated monograph by Siebenmann, which appeared in 1894.<sup>2</sup>

The drawings for the two lithographic plates illustrating this article were made by Leonard H. Wilder; the other drawings were made by Edith Capps Shambaugh. All the drawings were done accurately from actual preparations, and under my direct supervision. I wish to take this opportunity to express my deep obligations to Dr. Lewellys F. Barker for his interest and encouragement in this work, which has been carried out in the Hull Laboratory of Anatomy of this University.

#### METHODS

In this work a method, introduced by Oswald Eichler, of making celloidin casts of the labyrinth, from preparations in which the blood-vessels had been previously injected, was chiefly relied upon. Such celloidin casts, when cleared in creosote, become perfectly transparent, except so far as the network of blood-vessels obstructs, and when studied through the stereoscopic microscope present a beautiful picture of the circulation in its entirety which is invaluable in determining the general scheme for the distribution of the vessels with their anastomosing and branching. In addition to this method, thick serial sections of the injected decalcified labyrinth were made, in order to make sure of some points the interpretation of which at first appeared doubtful from the study of the celloidin casts. The injections were made into the embryo pig, in most cases while the animal was still warm. Material allowed to cool and stiffen did not give the most satisfactory results. Injections were made of pigs from the foetus at full term, measuring from 28 cm. to 30 cm., to embryos measuring 2.5 cm. in length. The injecting fluid used was a saturated aqueous solution of Prussian blue.

<sup>1</sup> *Anatomische Untersuchungen über die Wege des Blutstromes im menschlichen Ohrlabyrinth*, Leipzig, 1892.

<sup>2</sup> *Die Blutgefäße im Labyrinth des menschlichen Ohres*, Wiesbaden, 1894.

This penetrates readily the finest capillaries, and with this solution complete injections can be obtained.

For all embryos above 6 cm. in length, injecting through the umbilical cord gave the most satisfactory results. One of the umbilical arteries was selected, into which a small glass canula was secured. The rest of the cord was then tied off with a thread. For the newborn pig successful injections were obtained by injecting into the carotid. Embryos less than 6 cm. long were injected through the heart or liver. A small pointed metal canula was plunged into the apex of the heart or into the substance of the liver. The amount of pressure necessary to fill the vessels of the embryonic labyrinth, and still not rupture their walls, was always a delicate question to decide. Static pressure was chiefly used and was more satisfactory than any other method. For embryos 18 cm. long to the foetus at full term measuring 28 cm., pressure of an elevation from 80 cm. to 1 meter gave the best results. This was ample for filling even the capillaries and the veins, and it seldom ruined the preparation by rupturing the vessel walls. For smaller embryos the amount of pressure was graduated, as well as could be judged, according to the size of the embryo. For those measuring 2 cm. to 4 cm., pressure of 3 cm. to 6 cm. was used.

The injected embryo was placed in Müller's fluid and allowed to harden for several weeks. The labyrinth was then removed from the head. This, in embryos over 18 cm. long in which ossification was already well advanced, was easily done, for the more hardened capsule of the labyrinth can be readily shelled out from its location in the skull. The smaller the embryo, the more difficult it becomes to shell out the labyrinth in this way. Before ossification begins, however, the cartilage of the labyrinthine capsule can be differentiated from the surrounding tissue, and by working carefully with a small forceps this tissue can be removed and the labyrinth finally shelled out entirely free. By this method the labyrinth was removed in a perfect condition from embryos measuring only 2.5 cm. in length.

In making the celloidin cast of the injected labyrinth and in getting rid of the bony capsule in the older embryos in which the capsule had become ossified, the method employed by Eichler and Siebenmann in working with the adult ear was followed. The technique was varied somewhat, however, so that the work could be carried on more rapidly. The following is an outline of the steps in the process:

The shelled-out capsule of the labyrinth, with the stapes removed, was prepared for imbedding in celloidin by remaining for twenty-four hours in each of the following strengths of alcohol: 60 per cent., 70 per cent., 80 per cent., 95 per cent., 100 per cent.; then for twenty-four hours in absolute alcohol and ether, equal parts. It was then left for twenty-four hours in each of the following strengths of celloidin: 4 per cent., 10 per cent., 16 per cent., 20 per cent. The celloidin was then allowed to harden about the object for several days in a small glass dish covered with a bell-glass. It was further hardened in 80 per cent. alcohol for several days. The celloidin was then

scraped completely away from the surface of the preparation, and the work completed by the following process:

(1) Preparation placed in commercial hydrochloric acid for twenty-four hours. (2) Washed in water and the softened bony capsule carefully removed in small fragments with delicate forceps. (3) Washed for several hours in water. (4) Placed in 95 per cent. alcohol for twenty-four hours. (5) Left in 98 per cent. alcohol for a few minutes. (6) Cleared for several days in creosote.

In working with younger embryos before ossification has set in, this method can be materially shortened. Here all that is necessary is to imbed the shelled-out capsule of the labyrinth in celloidin, and after the celloidin has hardened under the bell-glass, as much of it as can be cut away without injuring the preparation is removed. The remaining block of celloidin containing the labyrinth is cleared up in alcohol and creosote as stated above. The clearing of such a preparation in creosote seldom requires more than twenty-four hours, provided it has been left in 95 per cent. alcohol for a sufficient length of time, from twenty-four to forty-eight hours.

The preparation is best left in creosote in a shallow dish where it can be moved about and examined from different view-points until it has been carefully studied and all doubtful vessels identified, when it can be permanently mounted in Canada balsam in a cell.

The stereoscopic microscope was found indispensable in studying these preparations. The perspective gained by the use of this instrument in examining the transparent casts of the labyrinth was necessary to place the vessels in their correct relations to each other.

Before entering upon the description of the circulatory system of the labyrinth, a word should be said regarding several difficulties that present themselves when the study of the injected celloidin cast of the labyrinth is undertaken. In the first place, the differentiation between arteries, veins, and capillaries in these preparations, where but one injecting medium was used, will at first be perplexing. This difficulty can be overcome by noting the character of the vessels, the intensity of the blue injection, and finally by tracing the vessels about which there is doubt to a large trunk, the character of which is easily ascertained. In a completely injected specimen where the arteries, veins, and capillaries are all filled, the arteries appear as blue-black lines, perfectly round and of smaller caliber than the corresponding veins. Through a considerable part of their course they, almost without exception, undergo to a marked degree a series of windings and convolutions. The veins appear as much paler, often considerably flattened bluish bands, and their course throughout is much straighter than the arteries, except as they approach the point where they break up into capillaries, when their course often becomes more irregular than the corresponding arteries. The veins never undergo convolutions as do the arteries. The capillaries in the greater part of the labyrinth present, in a perfectly injected preparation, a mesh-

work in which each individual loop can be traced. This is especially clearly defined in the vestibule and semicircular canals, except in the regions of the maculae and cristae acusticae, where the network is too dense to allow such close analysis.

A problem which is also perplexing at first is to determine which vessels are in the membranous labyrinth and which are in the osseous labyrinth or perilymphatic structures. This question can be definitely settled by a careful study of the preparations. Great assistance in determining the exact outlines of the membranous labyrinth in the vestibule and semicircular canals is found in the study of preparations that have never been cleared in creosote, but have been left in 95 per cent. alcohol for several days. Such preparations become partially transparent, and the outlines of the utricle and the membranous ampullae and semicircular canals are clearly defined. Another method that will assist in determining the outlines of the membranous labyrinth is by clearing the celloidin cast in acid-glycerine.<sup>3</sup> In preparations that have been thoroughly cleared in creosote, the utricle and the membranous ampullae and semicircular canals are definitely outlined in the incasing network of capillaries, and when viewed through the stereoscopic microscope their exact outlines can be traced.

#### THE ARTERIES

A single vessel, the labyrinthine artery, supplies the labyrinth of the ear, sending branches to the vestibule and semicircular canals, as well as to the cochlea. It reaches the labyrinth through the meatus acusticus internus, lying upon the auditory nerve. In this canal the labyrinthine artery breaks up into a number of branches. The first to be given off is a large vessel which follows along the ramus utriculo-ampullaris to the anterior surface of the vestibule, and is called the anterior vestibular artery (Plate V). The labyrinthine artery then divides into two trunks which almost immediately break up into a number of branches. These vessels are wound part way around the stem of the nervus cochleae, as if carried around it by the spiral growth of the cochlear tube (Plate VI). The branches from the two terminal trunks of the labyrinthine artery anastomose freely with each other, and from the anastomotic loops thus formed are given off the arteries which supply the cochlea, and others which, together with the anterior vestibular artery, supply the vestibule and the semicircular canals.

1. *The arterial tree of the cochlea.*—In the adult labyrinth and in the labyrinth of the foetus at full term the arteries undergo a remarkable series of convolutions described by Schwalbe as "glomeruli." These convolutions make it quite impossible in many areas of the cochlea to follow the vessels with accuracy. In the earlier embryonic stages the arteries do not possess this character, but run their entire course as practically straight vessels, free from spiral windings. This is true for all embryos of the pig measuring less than 12 cm. At about this age the arteries begin to develop much more rapidly than the surrounding structures, and, as a result, are thrown into these convolutions which, before the embryo reaches 16 cm. in length, are already well pronounced.

<sup>3</sup> HCl 2.5, glycerine 100.

To get a clear picture of the arterial tree of the cochlea, it is only necessary to study the circulation in the labyrinth of the embryo before the vessels have assumed their tortuous character. This can best be done in the pig from embryos measuring about 11 cm. Preparations where the injecting fluid has filled the arteries but where the capillaries and veins are practically empty, when viewed through the stereoscopic microscope, give a clear picture of the arterial supply of the cochlea, in which each vessel-trunk, each anastomotic loop, and each terminal branch can be traced. Such a preparation from the right ear of an embryo pig measuring 11 cm. is represented in Plate VII.<sup>4</sup> For the sake of clearness the cochlear spiral is represented as having been partly drawn out. With the exception of this slight distortion, each loop and each branch of the arterial tree has been drawn exactly as found. A correct idea can be obtained from this preparation of the scheme of distribution for the arteries of the cochlea, the details of which, however, in regard to the number and character of the anastomotic loops, differ materially for each individual labyrinth. To avoid confusion, the branches that supply the lamina spiralis in this preparation are not represented in the drawing.

Plate IX represents a preparation of the basal coil of the cochlea viewed from above, taken from the right ear of an embryo measuring 11 cm. The anastomotic arcades are seen to the best advantage from this view. Plate X gives the same view of the basal coil from a foetus at full term. The most striking difference between these two preparations is the convoluted character of the arteries in the latter, which to a certain extent obscures the arcade formation so clearly seen in the younger embryo. Also, the number of the anastomotic loops found in the younger embryo seems to have diminished somewhat before the embryo has fully developed.

The scala tympani at the proximal end of the basal coil is much larger than the scala vestibuli, and thus encroaches more upon the modiolus than does the scala vestibuli. This character of the scalae of the basal coil is most marked at its beginning, and disappears entirely before the first coil is completed. It is over this broadened part of the scala tympani at the beginning of the basal coil that the arcades are the most pronounced (Plates VII and IX). Near the point where the scala vestibuli begins to arch upward, that is, at the base of the lamina spiralis ossea, branches are given off from the arcades which follow along the lamina spiralis to supply this structure (Plate X). At the same point another set of branches arises, which, after forming an occasional arcade, arch over the top of the scala vestibuli, and ultimately break up into three distinct capillary areas, one over the scala vestibuli, one in the ligamentum spirale, and the third in the stria vascularis of the cochlear duct, in the manner to be described below. The straight character of the vessels radiating over the scala vestibuli, as found in the earlier embryos, is shown in Plates VII and IX, while the convoluted character assumed by these vessels before the embryo has fully

<sup>4</sup>It will be noted that the cochlea of the pig's ear has three and a half coils instead of two and a half, as in the human ear.

developed is shown in Plates V and X. The number of these vessels radiating over the scala vestibuli in the pig is about sixty-five, thirty of which supply the basal coil. The number described by Siebenmann for the human ear is thirty to thirty-five, of which fifteen go to the basal coil.

The upper coils of the cochlea are supplied by several large trunks from the arcades at the base of the cochlea, which pass up through the modiolus (Plates VII and IX). Branches from these trunks anastomose with each other, forming a spiral system of vessels with occasional well-developed arcades. This system follows closely along the coil lying near the base of the lamina spiralis ossea—an area which was called by Schwalbe the tractus spiralis arteriosus (Plate VII). From this spiral system of vessels branches are given off which radiate over the scala vestibuli at regular intervals, as in the basal coil. The arterial supply to the lamina spiralis of a coil above the basal turn of the cochlea, on the other hand, comes only in part from the tractus spiralis arteriosus of that coil. Larger or smaller areas are interspersed where the arteries to the lamina spiralis of a coil comes from the spiral system of vessels of the coil next below (Plate XI, Fig. 1). A similar character was also found in the veins which drain the lamina spiralis of the upper coils.

2. *The arterial system of the vestibule and semicircular canals.*—The arteries for these structures come, in part, from the anterior vestibular artery, which, as has been seen, is the first branch from the labyrinthine artery given off in the meatus acusticus internus. This vessel reaches the vestibule by following along the anterior surface of the ramus utriculo-ampullaris. Its first branch turns back along the anterior surface of the vestibule and, in the younger embryos at least, anastomoses freely with an artery from the anastomotic arcades at the base of the cochlea. The anterior vestibular artery supplies the macula acustica utriculi and the anterior crura of the superior and lateral semicircular canals and their ampullae (Plate V).

Another artery called the posterior vestibular artery springs from the anastomotic arcades at the base of the cochlea, and, crossing over the posterior surface of the vestibule just beneath the nervus ampullaris posterior, is distributed to the ampulla of the posterior semicircular canal and to the posterior crura of the posterior and lateral semicircular canals (Plate VI).

The crus commune is supplied by a single artery which is sometimes a branch from the posterior vestibular artery, sometimes it springs as an independent vessel from the arcades at the base of the cochlea, and crosses over the middle of the posterior surface of the vestibule lying to the anterior side of the aquaeductus vestibuli (Plate VI), and in a few cases it comes from the anterior vestibular artery.

#### THE VEINS

The circulation of the labyrinth is peculiar in having the exit for the veins at a point distinct from that through which the arteries enter. In the labyrinth of the pig's ear all the veins are collected into one large trunk, the vena canaliculi cochleae.



In embryos between 4 cm. and 6 cm. in length, however, there were sometimes found several veins which left the labyrinth with the aquaeductus vestibuli; but in the later stages, and in the foetus at full term, these veins had disappeared, and only the vena canaliculi cochleae remained to collect the blood from the entire labyrinth. This differs from the condition usually described for the human ear, where a large part of the venous blood from the utricle and semicircular canals is collected into veins and finds its exit along with the aquaeductus vestibuli. Schwalbe and Siebenmann describe venous trunks in the meatus acusticus internus of the human ear which are supplied in part by veins from the labyrinth. Eichler denied the existence of such veins. In the pig's ear no venous trunks of this kind were found.

1. *The venous tree of the cochlea.*—The blood from the ligamentum spirale of the basal coil is collected by a series of veins with broom-like origins which converge toward the middle of the under surface of the basal coil. These veins for the first half of the coil are collected into a large trunk which often follows along the middle of the under surface of the basal coil to empty into the vena canaliculi cochleae in the manner shown in Plate VI. The vein that collects the blood from the proximal end of the ligamentum spirale is a long vessel that begins where the ductus reuniens (Hensen) joins the ductus cochlearis. Lying first on the anterior surface of the vestibule, it runs forward around the anterior margin of the fenestra cochleae, where it takes a sharp turn backward to the under surface of the basal coil to join the venous trunk near the canaliculus cochleae (Plates V and VI). The veins that collect the blood from the ligamentum spirale of the second half of the basal coil empty into a large vessel called the posterior spiral vein. This vein is made up of two divisions which take their origin at about the junction of the middle and the distal thirds of the basal coil, and run in opposite directions around the coil, lying close along its posterior margin (Plates VI and VIII). At their point of origin anastomotic loops connect these divisions both above and below the coil. Near the beginning of the basal coil these two parts of the posterior spiral vein meet, and the common trunk thus formed empties into the vena canaliculi cochleae (Plate VI).

The scala vestibuli is provided with veins which begin in the capillary area over this scala (Plate X), and run downward and inward toward the modiolus. At the base of the lamina spiralis ossea these veins are joined by others from the lamina spiralis, and the common trunks thus formed take a direction downward and backward to join the posterior spiral vein (Plate X, and Plate XI, Fig. 1). Anastomotic loops are occasionally found between branches from these venous trunks. For that part of the cochlea in which the upper coils are applied closely to the coil beneath, that is, for all but the beginning of the basal coil, the veins which drain the scala vestibuli are joined by the veins from the scala tympani of the coil next above (Plate XI, Fig. 1). Into these common trunks are emptied, as a rule, the veins from the ganglion spirale and from the lamina spiralis of the upper coil. The manner in which these veins are collected into a large vein running through the center of the

modiolus is best seen in Plate VIII. An anterior spiral vein running along the inner margin of the scala vestibuli near the base of the lamina spiralis ossea, as described by Siebenmann for the human ear, does not exist in the cochlea of the pig's ear. Its place in the basal coil is supplied by the converging branches of the posterior spiral vein, and in the upper coils by the branches converging into a central venous trunk (Plate VIII).

The veins from the upper coils of the cochlea are collected in a central trunk which runs through the modiolus and empties usually into the posterior spiral vein in the first half of the basal coil (Plates IX and X). Or it joins that division of the posterior spiral vein which collects the blood from the distal end of the basal coil (Plate VIII). The manner in which the tributaries which join this central trunk collect the blood from the upper coils has already been described on p. 10, and is best seen in Plate VIII, and Plate XI, Fig. 1. The first tributary of this central trunk collects the veins from the upper coil of the cochlea, and follows the spiral direction of the coil, as does the artery for that part.

2. *The venous system of the vestibule and semicircular canals.*—The veins of the vestibule and semicircular canals are collected into two large trunks which empty into the vena canaliculi cochleae, either as separate vessels or after uniting into a common trunk (Plate VI). One trunk, called the anterior vestibular vein, follows closely the distribution of the anterior vestibular artery, and collects blood from the anterior surface of the vestibule, from the macula acustica utriculi, and from the anterior crura of the lateral and superior semicircular canals and their ampullae (Plate V) in the manner to be described on p. 15. The anterior vestibular vein begins where the ramus utriculo-ampullaris enters the vestibule. It runs forward and medialward across the anterior upper wall of the vestibule, curving over the recessus sphaericus to join the vein of the aquaeductus cochleae, either as a separate vessel or after joining the posterior vestibular vein.

The other large venous trunk of the vestibule is the posterior vestibular vein. This vein follows the distribution of the posterior vestibular artery. Its first tributary drains the capillaries over the posterior surface of the membranous ampullae of the superior and lateral semicircular canals, and crosses the vestibule by running in front of the crus commune. The posterior vestibular vein receives the blood from the crus commune, from the posterior crura of the lateral and posterior semicircular canals, and from the posterior ampulla. It follows the course of the posterior vestibular artery, lying directly beneath the nervus ampullaris posterior. It crosses the posterior surface of the vestibule, from the base of the posterior ampulla, to empty into the vena canaliculi cochleae (Plates V and VI). About midway across the vestibule it receives a large branch that runs transversely across the posterior surface of the vestibule, at right angles to the main vein. This transverse vein collects the blood from the posterior surface of the vestibule and from the macula acustica sacculae.

## THE VESSELS OF THE MEMBRANOUS LABYRINTH

A description of the blood-vessels of the membranous labyrinth is, in a large measure, a description of the capillary areas of the labyrinth. The distribution of the vessel trunks that have thus far been described is limited, for the most part, to the structures of the so-called osseous labyrinth, which is thus seen to serve as the means for carrying blood-vessels which break up into capillaries only when the membranous labyrinth is reached. The chief capillary areas found outside the membranous labyrinth are the capillaries over the scala vestibuli (Plates V and X) and those found among the nerves in the modiolus.

1. *The ductus cochlearis*.—The vascularization of the ductus cochlearis is limited, in the pig, to its lower and outer walls, while the upper wall, formed by the membrane of Reissner, is entirely lacking in blood-vessels. In the embryo of a calf measuring 70 cm., which was examined, Reissner's membrane was found well supplied with capillaries which communicated with the vessels of the lamina spiralis and also with those near the outer attachment of the membrane. Middendorp<sup>5</sup> found vessels in this membrane in young calves and dogs as well as in newborn children. Rüdinger<sup>6</sup> has pictured such vessels in the adult human ear. Neither Eichler nor Siebenmann found blood-vessels in Reissner's membrane of the human ear. The floor of the ductus cochlearis, formed by the lamina spiralis and the basilar membrane, is supplied by arteries which radiate from the modiolus outward, along the lamina spiralis ossea. The arteries leave the arcades in the modiolus as described on p. 8, and run out along the lamina spiralis ossea (Plates X and XI, Fig. 1). The veins from the lamina spiralis of the basal coil are collected in trunks which, uniting with the veins from the scala vestibuli, finally join the posterior spiral vein as described on page 10. In the basal coil the first set of capillaries formed from the vessel of the lamina spiralis is the network distributed in the ganglion spirale. This ganglion, in the proximal end of the basal coil, is lodged well out along the under surface of the lamina spiralis ossea, lying above the scala tympani (Plates VI and X). The vessels to the ganglion spirale of the basal coil shoot downward at right angles to the main trunks of the lamina spiralis and break up into capillaries in the ganglion (Plates X and XI, Fig. 1). In this network, when viewed directly from above or from below, can occasionally be seen small vessels which, for a considerable distance, follow the spiral direction of the coil.

The second capillary area formed from the vessels of the lamina spiralis lies in the crista spiralis. Small vessels leave the trunks near the base of Reissner's membrane, to pass upward and outward into the crista spiralis (Plate XI, Fig. 1), where they break up into loosely arranged, wide-meshed capillary loops, which, when viewed from above, are seen to be elongated somewhat in the spiral direction of the coil. These elongated loops in cross-section give the appearance of spiral vessels and may be readily mistaken for such vessels. The question as to the existence of blood-vessels in the crista spiralis

<sup>5</sup> Cited by EICHLER, *loc. cit.*

<sup>6</sup> *Atlas des menschlichen Gehörorgans*, München, 1886.

has been a subject for contention since the writings of Hushke in 1835. Most of the older writers found blood-vessels in this structure. Voltolini and Schwalbe describe the presence here of spiral vessels. The vessels in the pig's ear, when studied from sections, correspond closely to the vessels described by Schwalbe.

Under the crista spiralis the arteries of the lamina spiralis break up into loosely arranged capillary loops which penetrate the entire thickness of the lamina spiralis and extend outward along the basilar membrane as far as the space under the tunnel of Corti. These loops become more compact under the organ of Corti. The existence of spiral vessels forming the outer boundary for the vessels of the lamina spiralis, that is, lying under the tunnel of Corti, has generally been accepted. Schwalbe described a spiral vessel which, beginning in the basal coil, continued to the top of the spiral. Two parallel spiral vessels have sometimes been described. Middendorp found at places three such spiral vessels. A great deal of confusion regarding the exact nature of these so-called spiral vessels arose from the method of studying the blood-vessels from sections, which would permit the vessels to be followed out for only short distances. In examining a large series of celloidin casts of the labyrinth of the pig's ear, a marked difference in the distribution of vessels in this area for different ears, and in different parts of the same ear, was found. In most of the preparations the terminal loops of vessels were arranged in such a manner that, while often one and sometimes two, or even three, lines were formed extending a certain distance around the coil, the presence of a spiral vessel, in the sense that Schwalbe described it, was not found. In many preparations, especially in the first half of the basal coil, the tendency for two parallel vessels to run under the organ of Corti for a considerable distance in a spiral direction was well marked. The character of the vessels as usually found in this area is shown in Plate X. The arterial twigs break up into capillary loops which, as a rule, extend outward no farther than the space under the tunnel of Corti. Here the capillary loops empty into veins which carry the blood back through the lamina spiralis to the modiolus. The definite boundary thus formed by the terminal loops and their anastomosis with each other make the vessels which form the outer boundary appear often as continuous straight lines which take the spiral direction of the tunnel of Corti (Plate X). The vessels of the lamina spiralis are usually described as having no connection with the vessels of the ligamentum spirale. Middendorp, however, found such a connection in the ear of the rabbit and of the calf. In the pig it is not uncommon to find small veins running at irregular intervals between the loops of vessels under the tunnel of Corti and the veins in the ligamentum spirale (Plates X and XI, Fig. 1). Such vessels were found in the terminal coil as well as in the basal coil. These vessels were by no means constant, and in many well-injected preparations no such vessels could be found.

No blood-vessels whatever were found in the membrane of Corti or in the organ of Corti. This coincides with the findings of previous writers on this subject.

In the outer wall of the ductus cochlearis are two distinct capillary areas, one in

the stria vascularis, the other in the ligamentum spirale. In the stria vascularis, the area lying between the attachment of Reissner's membrane and the prominentia spiralis, is found a system of capillary loops which are said to lie in the epithelium lining the ductus cochlearis. This system extends from the beginning of the ductus cochlearis to its termination in the cupula cochleae. The arterial supply for this area is brought through an occasional twig from the arteries radiating over the scala vestibuli, while an occasional branch to the veins of the scala tympani carries the blood away. These vessels usually join the capillary loops near the center of the stria vascularis. The character of the capillary loops of the stria vascularis is shown in Plate XI, Fig. 2. The loops are elongated, extending usually in the spiral direction of the coil. The lateral boundaries are quite definitely limited, so that the vessels have more or less the character of continuous spiral vessels, comparable, to a certain extent, with the so-called spiral vessels of the basilar membrane.

The ligamentum spirale is freely provided throughout its entire thickness with capillary loops. These capillary loops extend into the crista of the ligamentum spirale in such a manner that, at the point where the basilar membrane is attached to the crista, terminal loops are found, similar to the terminal loops in the basilar membrane, and at places form a vessel which, for a longer or shorter distance, takes a spiral course along the crista. The arterial supply for these capillaries comes through the arteries which radiate over the scala vestibuli, while the venous blood is collected by the veins of the scala tympani. It is thus seen that the arteries radiating over the scala vestibuli supply, as has already been stated, three distinct capillary areas: first, the capillary area over the scala vestibuli; second, the capillaries in the stria vascularis; and third, the vessels in the ligamentum spirale. The first of these areas is located in the osseous labyrinth, while the last two are located in structures that are parts of the membranous labyrinth.

2. *The sacculus.*—The blood vessels of the sacculi are especially well developed in the region of the macula acustica sacculi. The arterial supply comes through several arteries which spring from the anastomotic loops at the base of the cochlea and run across the recessus sphaericus on the posterior wall of the sacculi. A number of very short stems are given off which turn forward toward the epithelial surface of the macula acustica sacculi. These break up into a flat matting of capillaries which presents a smooth surface toward the cavity of the sacculi. The venous blood from this area is collected by branches from the transverse vein of the vestibule, which in turn empties into the posterior vestibular vein (Plate VI).

3. *The utriculus.*—The utricle is surrounded by a loose capillary network, and in preparations that have been well injected and where the celloidin cast has been thoroughly cleared in creosote, the exact outlines of this membranous sac can be accurately distinguished by these incasing vessels. In such specimens the utricle is seen as a narrow elongated chamber which is crowded close to the posterior part of the vestibule into the recessus ellipticus. The conspicuous feature of the blood-supply to

the utricle is, however, the vascularization of the macula acustica utriculi. The arterial supply for this area comes exclusively from the anterior vestibular artery through several small branches which run along the anterior surface of the utricle, and, as in the case of the vessels to the macula acustica sacculi, send off a number of short stems which turn abruptly toward the epithelial surface and divide into a dense capillary matting. This capillary network lies in the anterior wall of the utricle and presents a flat, smooth surface toward the cavity of the utricle. The venous blood is collected by tributaries of the anterior vestibular vein.

4. *The ductus endolymphaticus*.—This membranous tube is incased by capillaries which are supplied by a small arterial twig, usually a branch of the posterior vestibular artery, and are drained by a small vein which empties into the transverse vestibular vein. In preparations where enough of the duct is preserved to show its flaring, trumpet-like opening into the sacculus endolymphaticus, a small dural vein can be seen which collects the blood from that end of the duct (Plate VI).

5. *The membranous ampullae*.—These structures receive their blood-supply from the following sources: the superior and lateral ampullae from branches of the anterior vestibular artery, the posterior ampulla from the posterior vestibular artery. The anterior vestibular vein collects the blood from the superior and lateral ampullae, and the posterior vestibular vein from the posterior ampulla and part of the posterior surface of the lateral ampulla (Plates V and VI). The exact form of each membranous ampulla is outlined by a network of blood-vessels spread over its surface. The arterial blood is supplied by one or two branches which approach the ampulla from its base. The venous blood is collected by several branches which, as a rule, take a course similar to that followed by the arteries. It was often found that, where a single arterial twig supplied the ampulla from one side, a vein would collect the blood from the dome of the ampulla and run back to its base on the opposite side (Plate XII, Fig. 1). Siebenmann, in his work on the blood-vessels in the labyrinth of the human ear, pictures in Plate III a condition in which a large vein arches over the dome of each ampulla and collects the blood by a number of short branches which extend down to the ampulla. This condition was not found in the labyrinth of the pig's ear. The cristae of the ampullae, like the maculae acusticae of the utricle and sacculus, are richly supplied by a dense matting of very finely woven capillaries (Plate XII, Fig. 1). The arteries enter the sulcus ampullaris and the veins emerge along with the nerve.

6. *The canales semicirculares*.—The plan of the blood-supply for the semicircular canals of the pig's ear corresponds in general to the distribution of vessels usually described for the human ear. Each canal is supplied with two arteries and two veins, one of each for each crus. The blood-supply for the several canals is as follows: The anterior crura of the superior and lateral semicircular canals receive each a branch from the anterior vestibular artery, while the venous blood is collected by a tributary of the anterior vestibular vein. The posterior crura of the lateral and posterior canals receive

each a branch from the posterior vestibular artery and a tributary of the posterior vestibular vein carries the blood away. The crus commune carries a single artery the origin of which is described on p. 9. This artery divides near the end of the crus into two branches, one of which goes to the posterior semicircular canal, the other to the superior. Two veins, one from each of these semicircular canals, unite at the end of the crus commune near the point where the artery divides. The common trunk thus formed follows the course of the artery through the crus commune, and empties into a branch of the posterior vestibular vein (Plates V and VI). This differs from the observations of Siebenmann on the human ear, where the veins from the posterior and superior canals run through the crus commune as separate vessels.

The semicircular canals were so perfectly injected in a number of the specimens that it was not only possible to follow the arterial and venous trunks throughout their entire course, but with the aid of the stereoscopic microscope every capillary loop throughout the canal could be traced and the character of these loops studied.

The artery for a time after it enters the canal usually clings close to the inner concave surface of the bony canal. It begins soon to gradually approach the membranous canal which lies close to the outer convex surface of the bony canal. The artery does not usually reach the membranous canal until near the center of the arch. Its terminal branch when it reaches the membranous canal breaks up immediately into capillary loops. The artery throughout the semicircular canal takes a course almost straight. It sends off from five to ten small branches which run out to supply the membranous canal (Plate XII, Fig. 1).

The veins which drain the crura of the semicircular canals follow, as a rule, close along the inner surface of the membranous canal, taking an irregular zigzag course. About the point near the center of the arch where the terminal branch of the artery breaks up into capillaries, the vein begins as a separate vessel. Thus for a short space in the center of the arch only the capillary loops which encase the membranous canal are to be found. Throughout its course the vein receives a number of small branches which in turn collect the blood from a network of loosely arranged capillary loops which surround the membranous canal. These capillary loops, when viewed from the convex surface of the arch of the semicircular canal, present a characteristic appearance. The loops come up on either side of the membranous canal, but do not completely surround it, for a zone is left along the middle of its convex surface free from vessels, except for an occasional branch which, crossing over the zone, connects the loops on one side of the membranous canal with those on the opposite side (Plate XII, Fig. 2).

The points of special interest in this description of the blood-vessels in the labyrinth of the pig's ear may be summarized briefly as follows:

1. The several divisions of the labyrinthine artery which go to supply the cochlea anastomose freely with each other through a number of anastomotic loops or arcades at the base of the cochlea, thus insuring for each part a blood-supply reinforced freely from each division.

2. The arterial supply to the cochlea is arranged on such a plan that, as a rule, the vessels which send out the arteries to supply the scala vestibuli of a coil send out another set of arteries which supply the lamina spiralis of the coil next above. The arrangement usually described for the human ear, where the arteries for the scala vestibuli and for the lamina spiralis of the same coil come from the same vessels, is found in the cochlea of the pig's ear, but only as the exception.

3. The venous blood of the cochlea drains entirely into the vena canaliculi cochleae. The veins from the ligamentum spirale of the first half of the basal coil are collected into a large trunk which runs along the middle of the under surface of the basal coil to empty into the vena canaliculi cochleae. The veins from the remainder of the basal coil are collected into the posterior spiral vein which runs along the posterior inner margin of the coil.

4. The venous blood from the upper coils of the cochlea is collected by a tributary of the posterior spiral vein. This vein in its beginning follows the spiral direction of the upper coil. It then passes directly downward through the modiolus to join the posterior spiral vein, receiving tributaries from the upper coils which converge toward this central vessel. The anterior spiral vein which Siebenmann found in the cochlea of the human ear does not exist in the ear of the pig.

5. The veins which lie between the coils of the cochlea are supplied by two sets of tributaries, one of which collects the blood from the scala vestibuli of the coil beneath; the other set collects the blood from the scala tympani of the coil above.

6. The so-called spiral veins of the cochlea, which are usually described as running under the tunnel of Corti, in the crista spiralis, in the crista of the ligamentum spirale, and in the prominentia spiralis, are formed in the ear of the pig from capillary loops which form the boundary line for distinct capillary areas in these parts.

7. There was often found in the cochlea of the pig's ear a connection between the vessels of the lamina spiralis and those of the ligamentum spirale. This connecting link consisted of straight veins which ran from the terminal loops under the tunnel of Corti across to the veins in the crista of the ligamentum spirale, and were found in the terminal as well as in the basal coil.

8. The arterial supply for the vestibule and the semicircular canals comes in part from the anterior vestibular artery, and in part from arteries which spring from the anastomotic loops between the arterial trunks which supply the cochlea.

9. The venous blood from the vestibule and the semicircular canals is collected into two large trunks which empty into the vena canaliculi cochleae. This is in striking contrast to the condition found by Siebenmann and Eichler in the human ear, where the veins from the semicircular canals left the labyrinth with the aquaeductus vestibuli.

10. The capillaries are distributed almost exclusively to the membranous labyrinth. In the semicircular canals this is shown the most clearly. Here the capillaries surround the membranous canal while the veins run along its inner concave surface,



and the artery, for the most part, clings to the inner concave surface of the osseous canal, sending an occasional twig to the capillary loops around the membranous tube.

11. The capillary loops of the membranous semicircular canals do not, as a rule, completely surround this tube, but leave a zone along its convex surface free from vessels except for an occasional connecting loop which runs across this space.

#### EXPLANATION OF PLATES

NOTE.—The arteries are in red, the veins and capillaries in blue. The explanations of Plates V and VI appear on page 20.

##### PLATE VII

The arterial tree of the cochlea from the right ear of an embryo measuring 11 cm. in length. The coils of the cochlea are represented as having been partially drawn out, in order to show better the branching and anastomosis of the arteries. The drawing is from a preparation in which only the arteries were injected.

- a, a.* The two arterial trunks of the cochlea.
- b, b.* Anastomotic loops at the base of the cochlea.
- c, c, c.* Tractus spiralis arteriosus.
- d.* Terminal branch of the arteries forming the tractus spiralis arteriosus.
- e, e.* Arteries radiating over the scala vestibuli.

##### PLATE VIII

The venous tree of the cochlea from the right ear of an embryo measuring 11 cm. in length. The coils of the cochlea are drawn out as in Plate VII. From a preparation in which only the larger veins were injected.

- a, a.* Vena spiralis posterior.
- b.* Anastomotic loop between the two branches of the vena spiralis posterior.
- c.* Veins which collect the blood from the lamina spiralis.
- d.* Veins which collect the blood from the scala tympani.
- e.* Central venous trunk, collecting blood from the upper coils.
- f.* Vein from terminal coil, following the spiral direction of the coil.
- g.* Vena vestibularis anterior.

##### PLATE IX

Basal coil of the cochlea from the right ear of an embryo measuring 10 cm. in length. Showing character of the anastomosis at the base of the cochlea in a young embryo before the arteries have become convoluted.

- a, a.* Arterial trunks of the cochlea.
- b.* Anastomotic loops.
- c, c, c.* Stumps of arteries which supply the upper coils.
- d.* Anastomotic loops of arteries lying over the scala tympani.
- e, e.* Arteries radiating over the scala vestibuli.
- f, f.* Vena spiralis posterior.
- g.* Stump of central venous stem which collects blood from the upper coils.
- h.* Vena vestibularis anterior.
- i.* Vena vestibularis posterior.

##### PLATE X

Basal coil of the cochlea from the right ear of a foetus at full term. A section of the scala vestibuli is removed to show vascularization of the lamina spiralis. At this age the arteries have assumed the characteristic convolutions of the adult labyrinth.

- a, a.* Arterial trunks of the cochlea.
- b, b.* Anastomotic loops of the arteries.
- c.* Arteries radiating over the scala vestibuli.
- d, d.* Vena spiralis posterior.
- e.* Stump of vein which collects blood from the upper coils.
- f.* Capillary areas of the scala vestibuli.
- g.* Vein on upper margin of fenestra cochleae.
- h.* Vessels of the lamina spiralis.
- i.* Capillaries of the ganglion spirale.
- j.* Terminal loops of blood-vessels lying under the tunnel of Corti.
- k.* Veins connecting vessels of lamina spiralis with those of the ligamentum spirale.

PLATE XI

FIG. 1.—A thick section through the center of the cochlea, one-half only being represented. Semi-schematic.

- a, a, a.* Ganglion spirale.
- b, b, b.* Crista spiralis.
- c, c, c.* Terminal loops of vessels under the tunnel of Corti.
- d, d.* Venous connection between vessels of the lamina spiralis and those of the ligamentum spirale.
- e, e.* Venous trunks lying between coils of the cochlea which collect blood from the scala vestibuli of the coil beneath, also from the scala tympani of the coil above.

FIG. 2.—Section from the basal coil of the cochlea. A piece is removed from the outer wall of the ductus cochlearis to show the blood vessels in the stria vascularis.

- a.* Blood vessels of the stria vascularis.
- b.* Ductus cochlearis.
- c, c.* Arteries radiating over the scala vestibula.
- d, d.* Veins of the scala tympani.

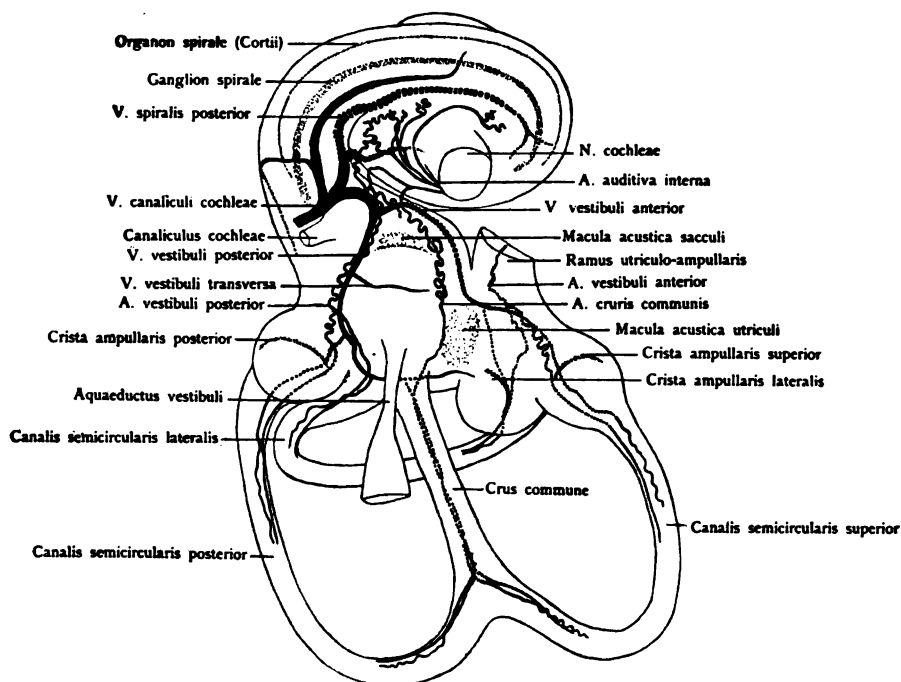
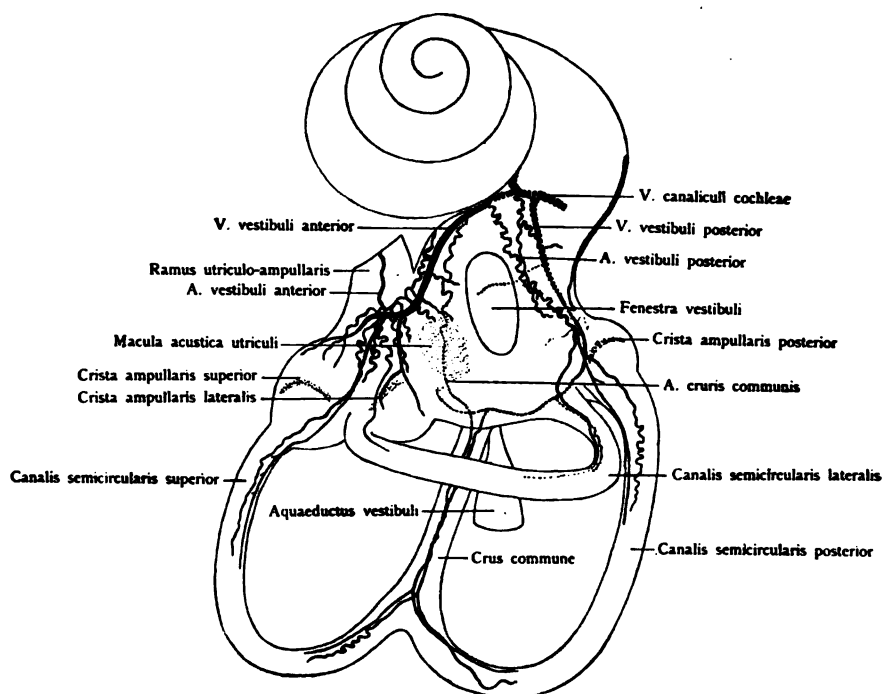
PLATE XII

FIG. 1.—Canalis semicircularis lateralis from the right ear of a foetus at full term. Viewed from above.

- a.* Ampulla ossea.
- b.* Ampulla membranacea.
- c.* Crista ampullaris.
- d.* Canalis semicircularis (osseus).
- e.* Ductus semicircularis (membranaceus).
- f, f.* Arteries.
- g, g.* Veins.

FIG. 2.—Section of the canalis semicircularis lateralis taken from near the center of the arch.

- a.* Canalis semicircularis (osseus).
- b.* Ductus semicircularis (membranaceus).
- c.* Artery.
- d.* Vein.
- e, e.* Capillary loops.
- f, f, f.* Vessels connecting the capillary loops on opposite sides of the ductus semicircularis.

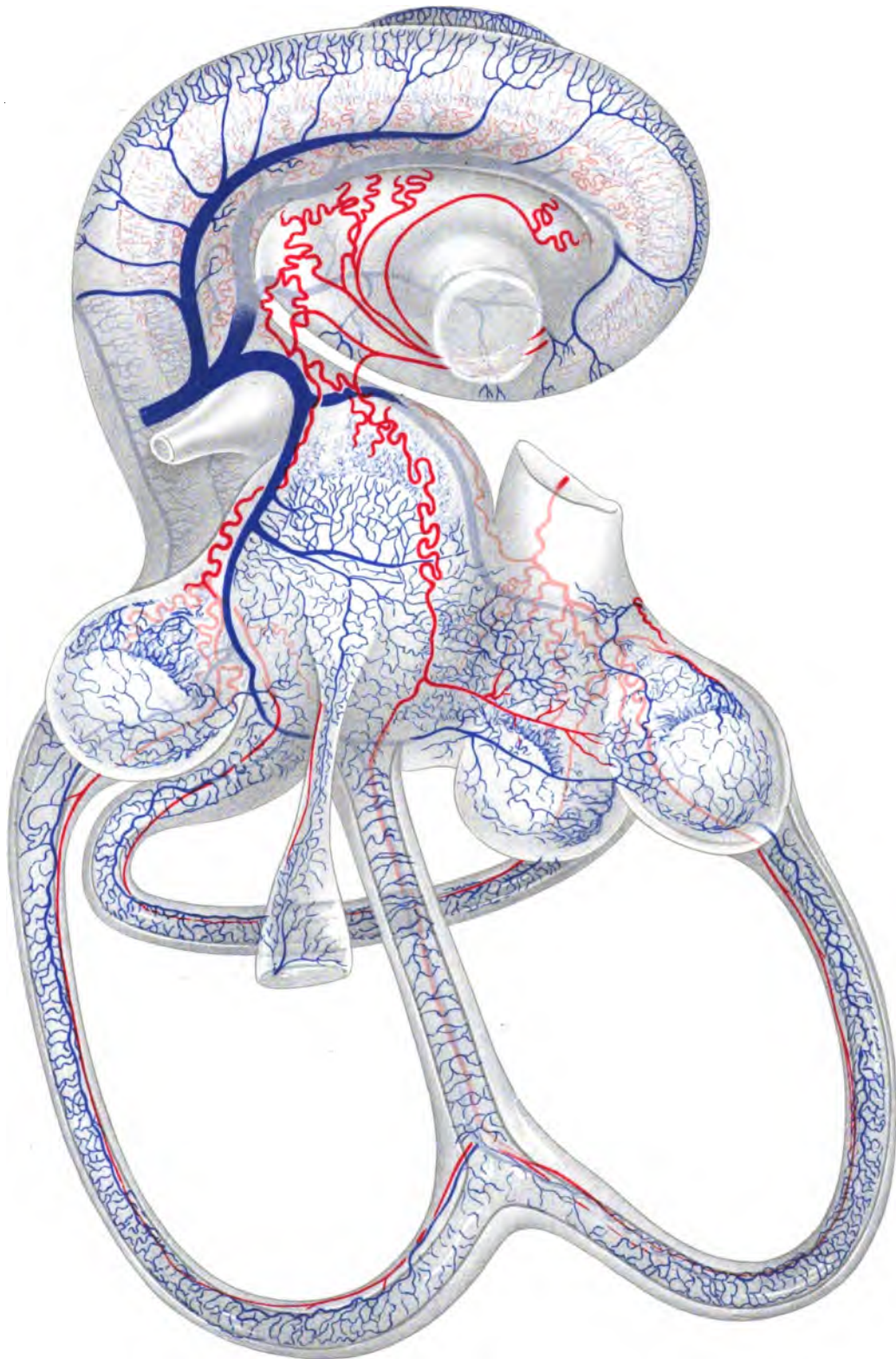


KEY TO PLATES V AND VI

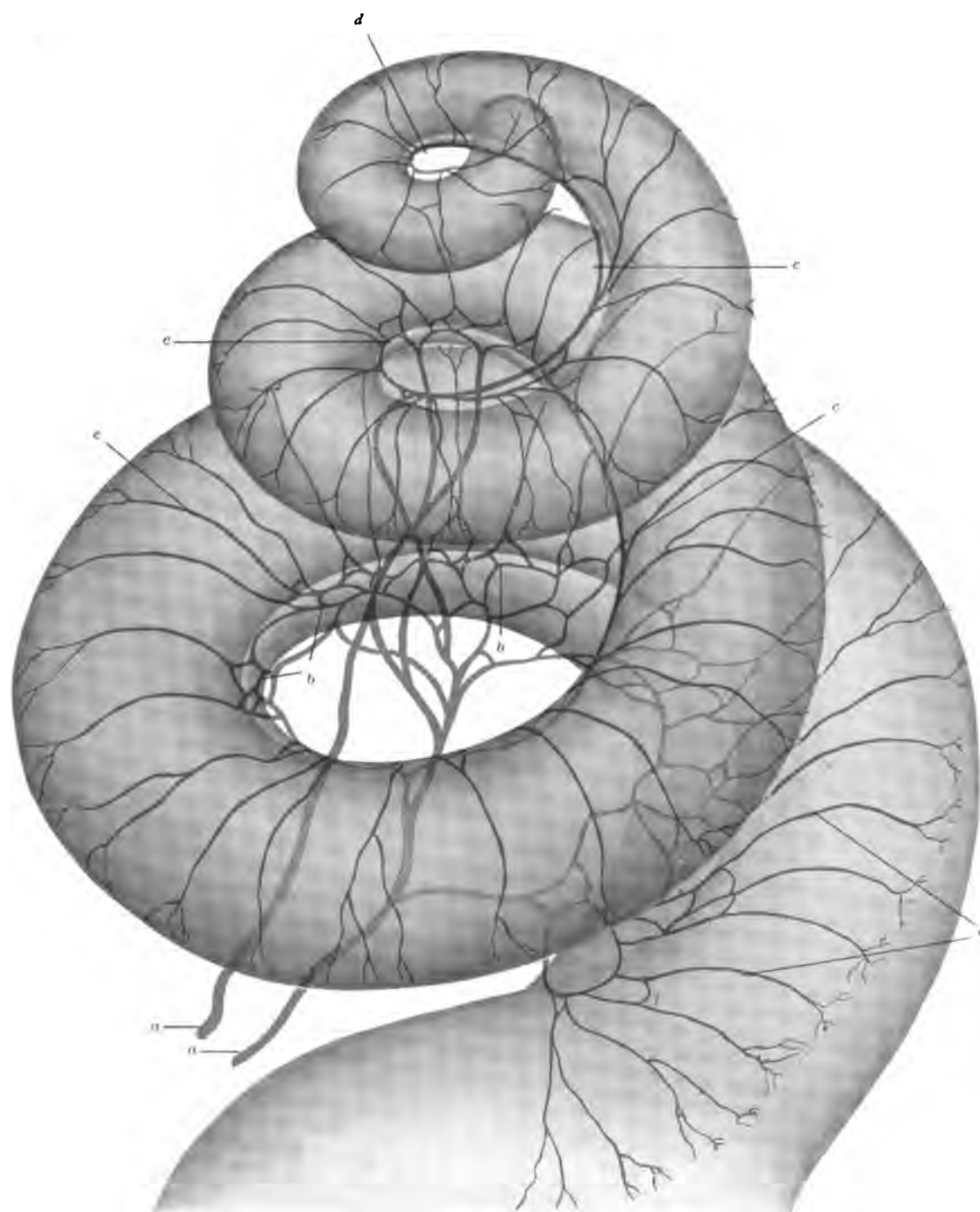






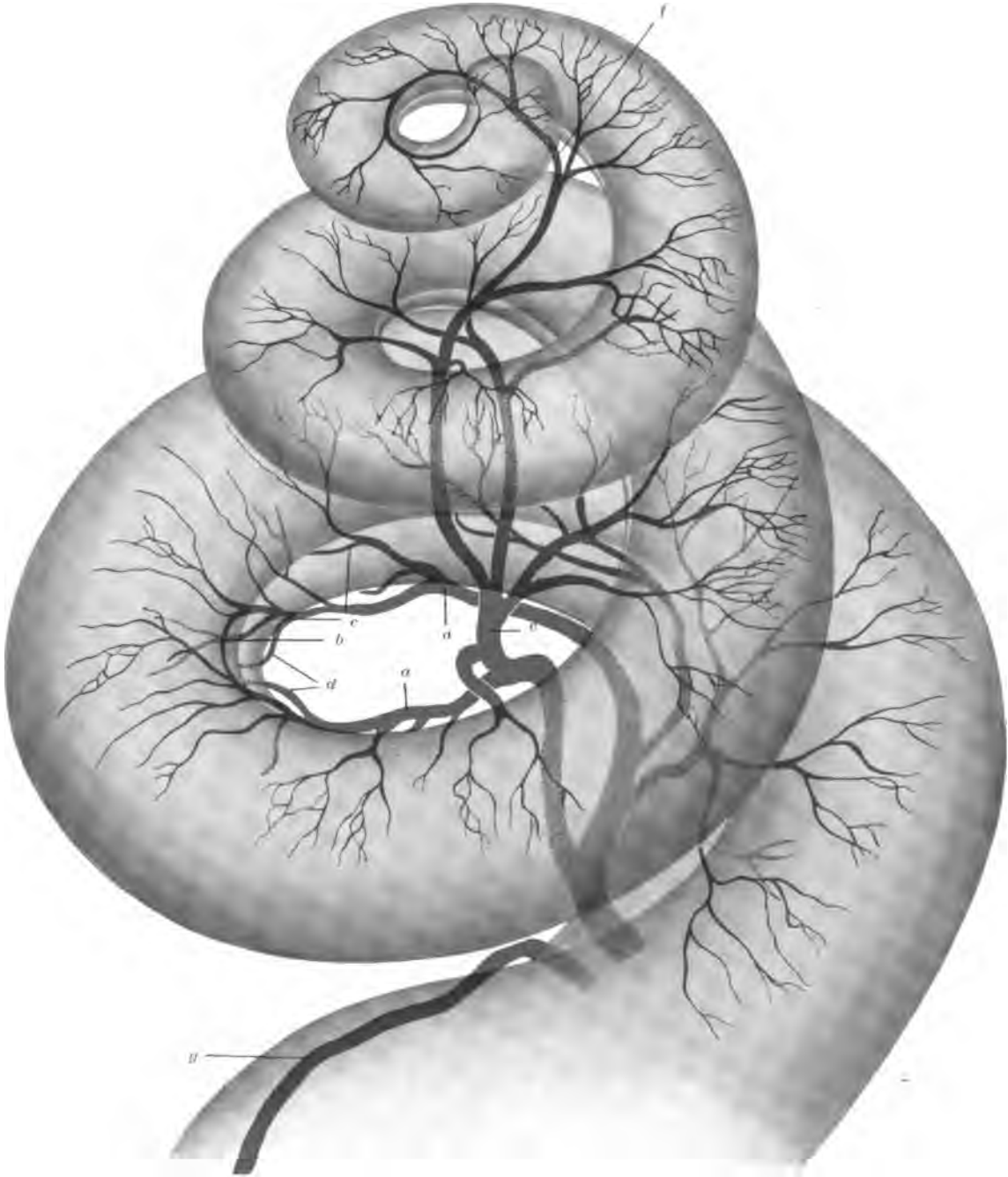




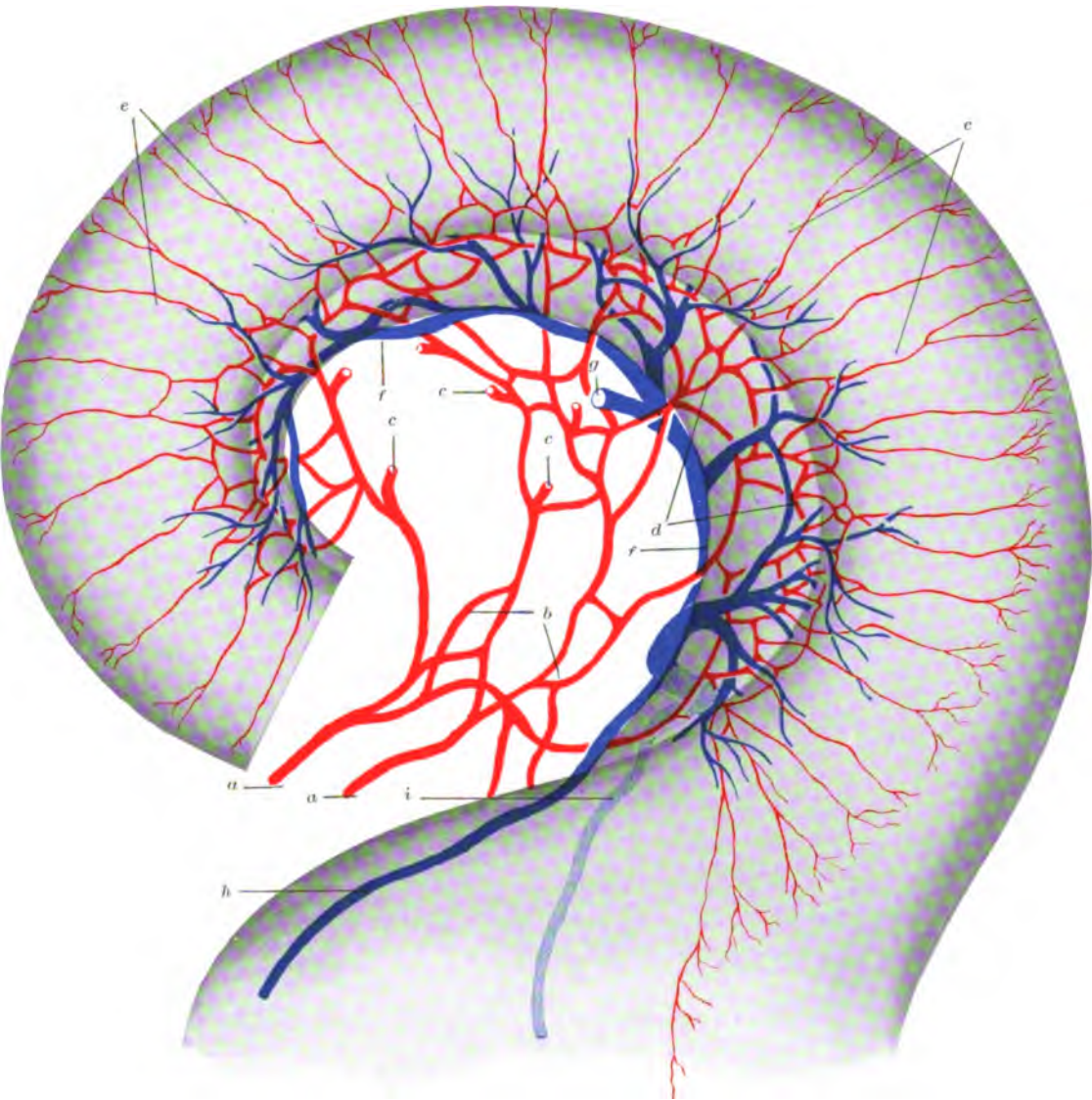




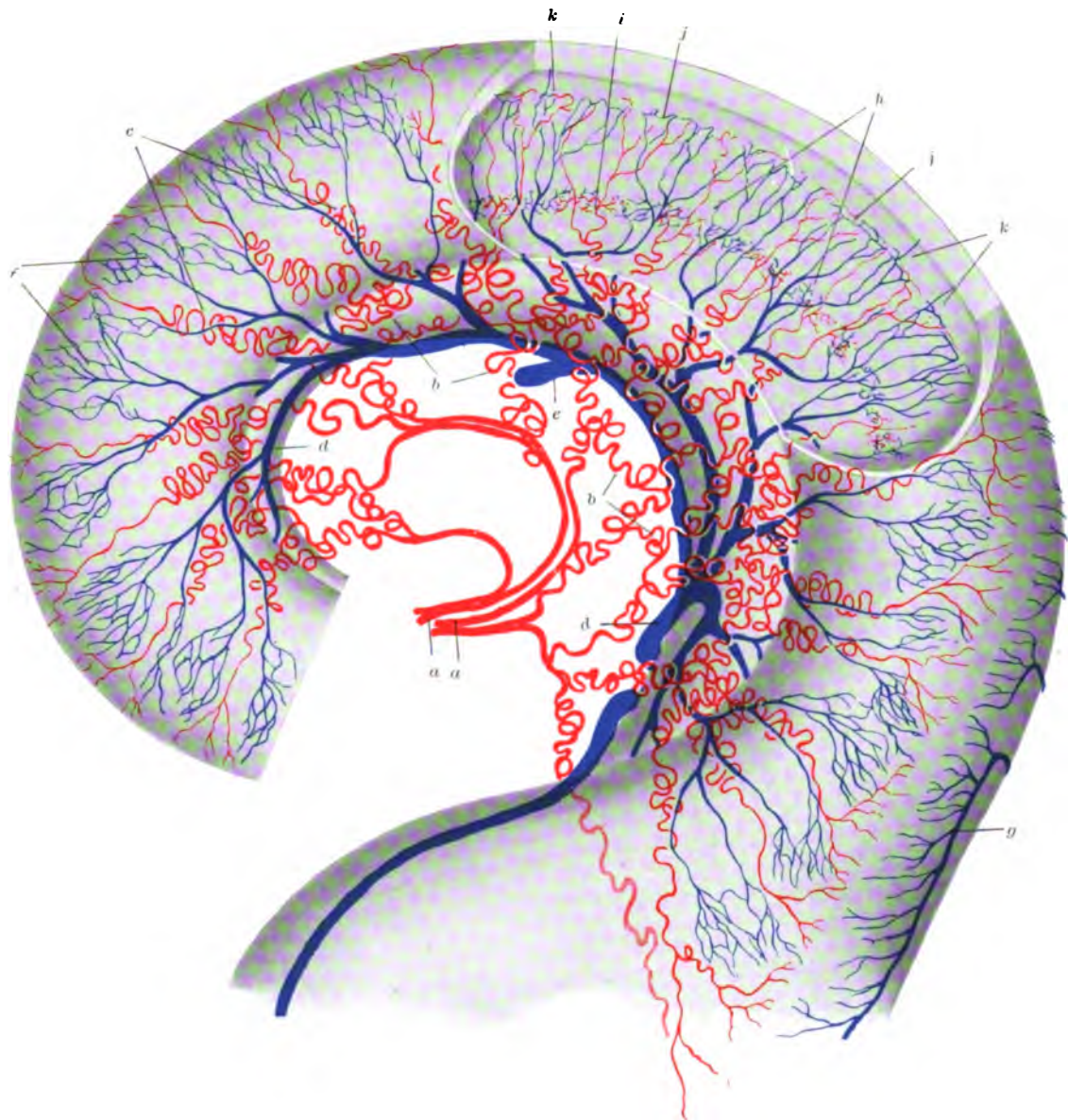














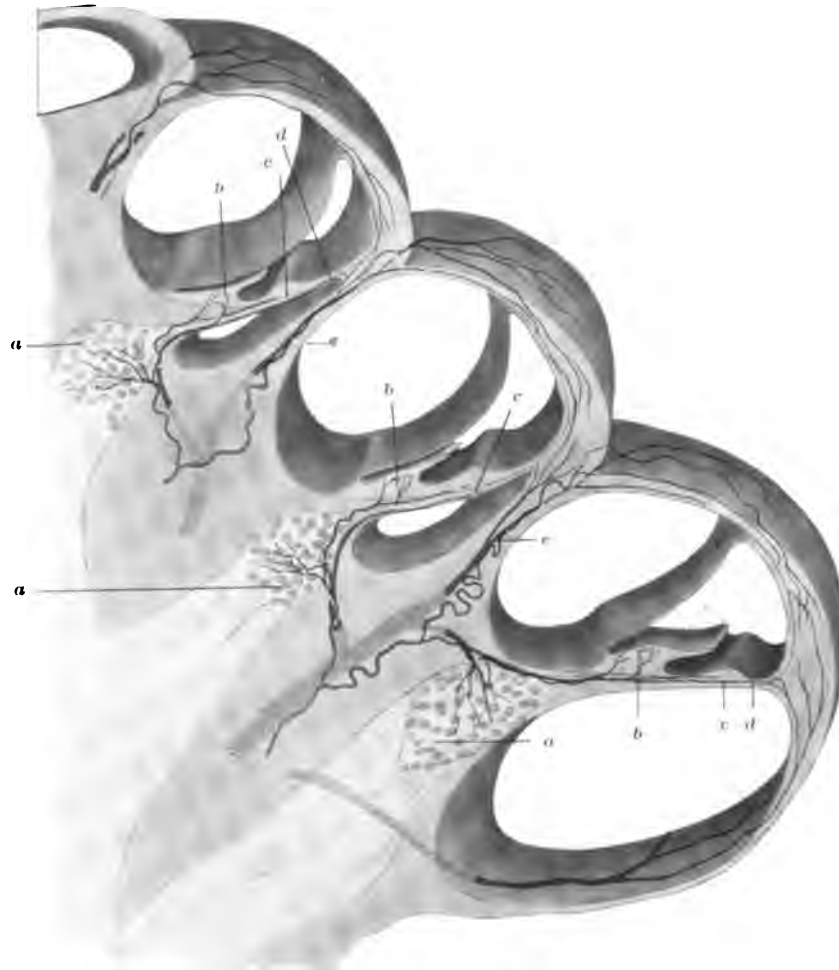


FIG. 1

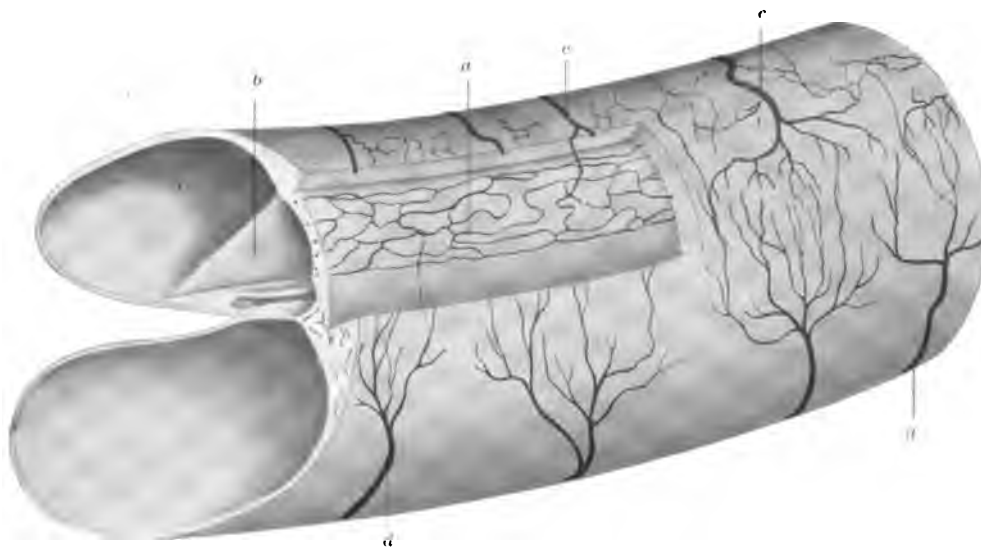


FIG. 2





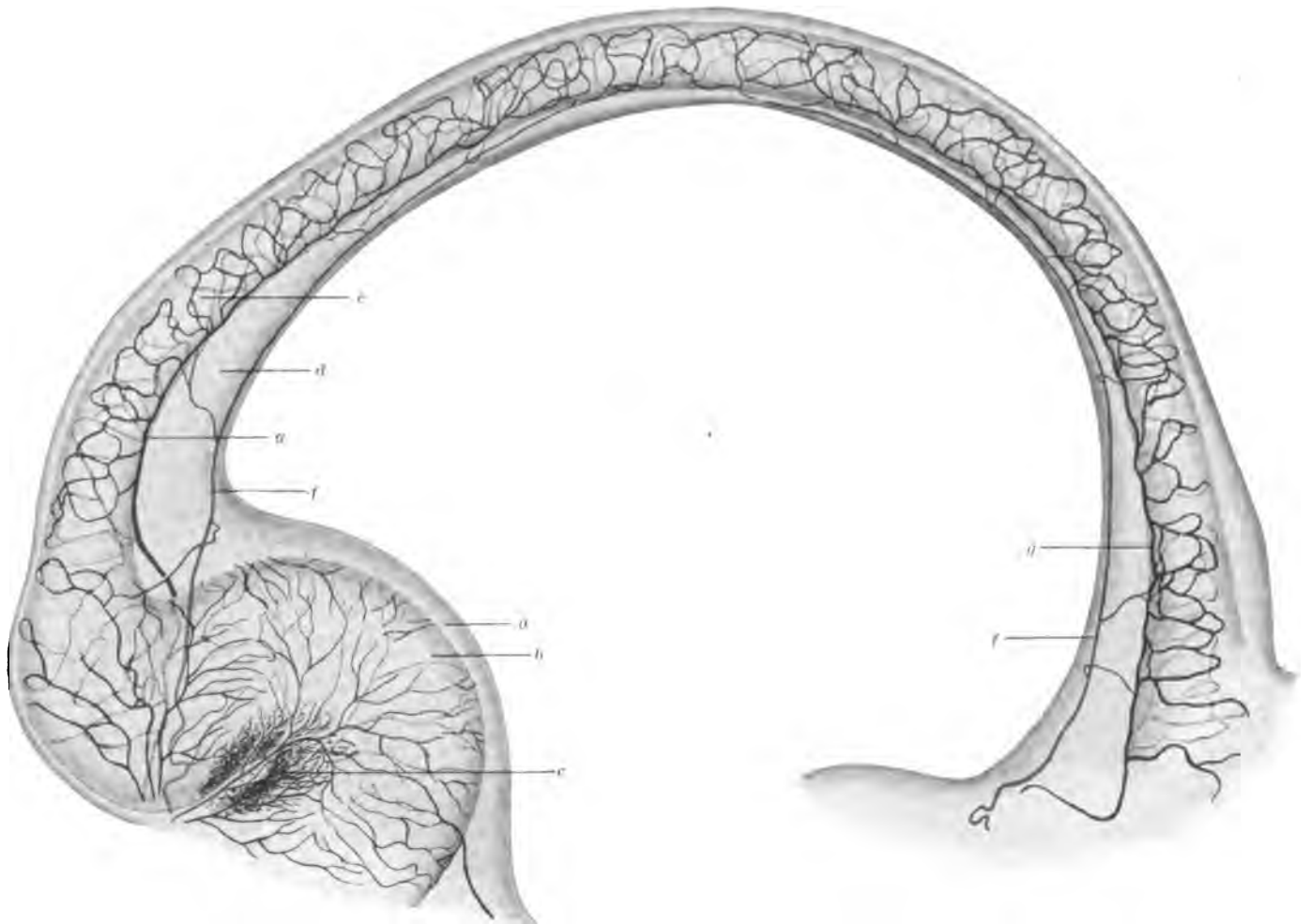


FIG. 1

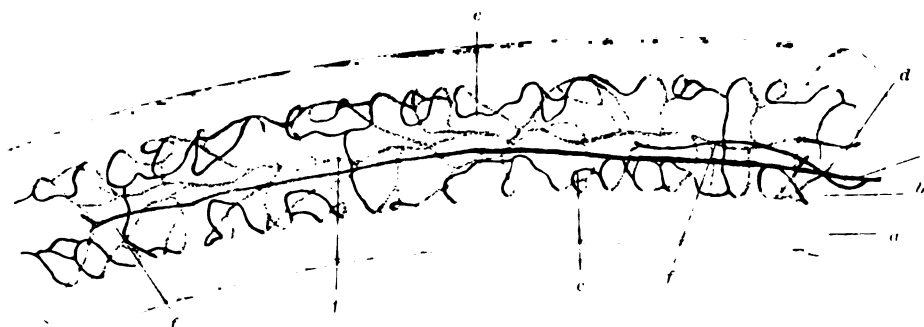


FIG. 2



**THE ANIMAL ECOLOGY OF THE COLD SPRING  
SAND SPIT**



# THE ANIMAL ECOLOGY OF THE COLD SPRING SAND SPIT, WITH REMARKS ON THE THEORY OF ADAP-TATION

C. B. DAVENPORT

COLD SPRING sand spit runs from the west shore of Cold Spring Harbor, Long Island, eastward to within 100 or 200 feet of the eastern shore of the harbor. The history of the formation of the spit is briefly this: Cold Spring Harbor (Fig. 1) is a fiord-like re-entrant about ten kilometers long, emptying at its lower or northern end into Long Island Sound about one-fifth of the way from Hell Gate at New York city to "The Race," south of New London. The Sound itself, 175 kilometers long by 35 kilometers broad at its widest point, and having a prevailing depth of about 30 meters, receives large streams of water from the north—the Connecticut, Housatonic, and Quinnipiac rivers, and many minor ones. These give it a low specific gravity, 1.020, and a muddy bottom. Long Island is covered over its northern part with glacial débris, forming hills that rise to a height of over 100 meters. Between these hills streams flowing north have cut valleys and, in the general sinking to which the whole area has been subjected, these valleys have become drowned, forming long, straight but shallow harbors of which Cold Spring Harbor is an excellent type (Shaler, 1902). Into the head of the harbor a small stream flows with a summer discharge of not far from five cubic meters per minute. This stream is dammed thrice along its course of two miles, so that the deposit that it carries into the basin above the beach is only fine mud. The effective winds at Cold Spring Harbor blow from the northeast and, striking with violence upon the bluffs on the west side of the harbor, tend to wear them away. The currents setting southward then carry this eroded material until it is dropped in the shallow and protected waters of the upper end of the harbor, where the sand spit or "beach" is now found. The inner harbor thus cut off is about 800 meters long by 600

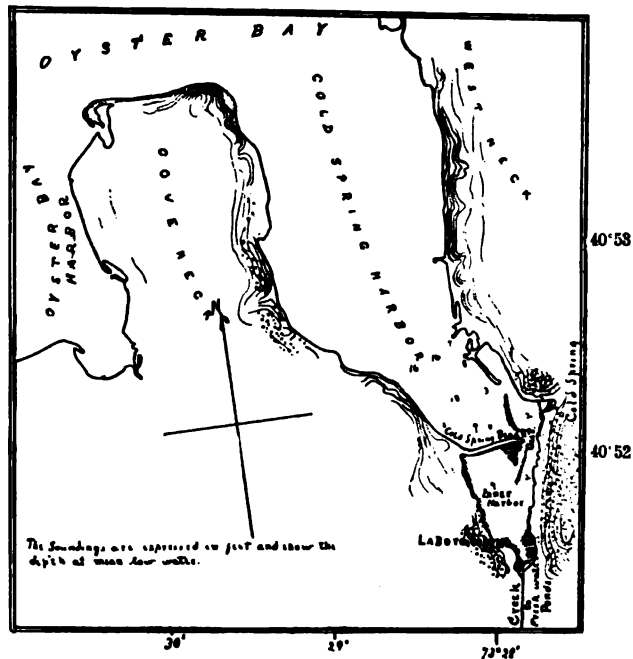


FIG. 1.—Map of Cold Spring Harbor, showing spit (Cold Spring Beach).

wide, and at mean high tide has a prevailing depth of less than 2.5 meters. Its sandy bottom is for the most part covered with half a meter of fine black mud. This is largely exposed at low tide, and supports an abundant growth of "sea cabbage" (*Ulva*). The specific gravity of the water at high tide varies from 1.018 near the entrance to the inner harbor to 1.005 at the surface near the inlet of the creek. Near the "gut" where the tides rush in and out of the inner harbor the bottom is kept scoured and consists of gravel and stones. Outside the sand spit the water has a pre-



FIG. 2.—View of Cold Spring Harbor at high tide looking north. The inner harbor is in the foreground; between it and the outer harbor is seen the sand spit. Close to the sand spit on the inside is a fringe of *Spartina polystachya*.

vailing specific gravity of 1.019 and is about 6 to 8 meters deep at high water. A bar (Fig. 2), almost submerged at high tide and consisting largely of hard mud, has formed opposite to the outer entrance to the gut and affords a "shallow sea" fauna.

The sand spit itself (Figs. 2 and 3) is 660 meters long and varies in width from 80 meters at its western end to 15 meters at its eastern end at high water. The prevailing width is about 40 meters. The highest part of the spit is about one meter above mean high tide, and the average height of tide is 2.4 meters. The slope is gradual on the outside (north side), it being 40 meters from high to low water at the western third of the spit. The slope increases to 2.4 meters in 15 at the eastern end. The material of which the spit is built is sand, which at the western end contains much gravel, apparently because of the greater carrying power of the surf, which is highest here. At the gut the sand is very coarse on account of the rushing tide. In the middle stretches of the beach the sand is finer. On the inner face of the spit mud is deposited. On account of the sandy beach and the currents and surf that are washing it all the time, no plants grow on the outside of the sand spit

between average tides. On the inside of the spit, on the other hand, the marsh grass, *Spartina*, finds abundant foothold. All these facts have an important influence on the distribution of the animals of the sand spit. Indeed, for the purposes of this account of the fauna of the spit it will be necessary to treat separately its outer and inner margins and its tip, just because the conditions are so different in the three situations.



FIG. 3.—View of sand spit taken near western end looking east. Terrestrial border zone with *Ammophila* in the foreground. The outer beach with the storm bluff, wreckage-strewn upper beach, and lower beach. At the right the inner beach, passing into mud flats at the edge of which *Spartina polystachya* is growing.

#### A. ANIMALS OF THE OUTER BEACH

The outer beach may, for our purposes, be divided into three zones which we may herein designate as : (1) the submerged zone; (2) the lower beach; and (3) the upper beach. The submerged zone includes all that portion of the beach that lies below mean low tide, but which may be exposed by the lowest spring tides combined with southerly winds (Fig. 4). It is a region that is normally covered with water; it is the very margin of the shallow sea. The lower beach is that zone which lies between mean low tide and mean high tide. It is the zone that is twice each day exposed to the air and submerged. It passes without any sharp break into the submerged zone (Figs. 4 and 5). The upper beach is limited on the one hand by the line of débris that marks the average high tide, and on the other by a bluff, half a meter high, that has been cut by storms (Fig. 5).



## I. FAUNA OF THE SUBMERGED ZONE

The animals that live below the high-tide line may be considered under four heads: (a) sessile species, (b) crawling species, (c) burrowing species, and (d) swimming species.

a. *The sessile species of animals*, inhabiting the lower beach, include certain molluscs, especially bivalves, or Lamellibranchiata. All the lamellibranchs feed on minute particles of organic matter: Algæ, Infusoria, and decaying bits of organisms.



FIG. 4.—Photograph of lower beach, north side of sand spit, near the eastern end, at very low tide. The gut is seen in the background. At the water's edge (submerged zone) is a dense growth of the alga *Enteromorpha*. The naked lower beach is thickly peopled with the mud snail, *Nassa obsoleta*, visible as dots in the photograph.

The silt brought down by the creek is rich in such material and the algæ thrive on the mud flats, consequently these flats and the shallow sea around the flats are especially favorable feeding-grounds for these molluscs. Here occur oysters (*Ostrea virginiana*) in a semi-domesticated state. They normally attach themselves to some solid object while still young, but those of Cold Spring Harbor have been mostly transplanted and lie loose in the shallow waters, but altogether incapable of movement or of any other defense than that provided by their two thick valves. Here also occurs the scallop (*Pecten irradians*), which attaches itself while yet less than two weeks old, in the middle of August. The attachment is chiefly to eel-grass or to small stones. About the middle of September the scallops migrate into the inner harbor to live on

the mud-flats like the oyster, but they never lose their power of free migration. The jingle shells (*Anomia simplex*) are permanently attached to stones or larger shells, such as *Pecten*, from early life. On the lower beach one also finds two species of *Arca* (*Arca transversa* and *Arca pexata*); the hen-clams, *Macra solidissima* and *Macra lateralis*; the hard clam, *Venus mercenaria*; and *Liocardium mortoni*—all lying on or imbedded in the muddy bottom. Here also are found certain species of lamellibranchs that burrow in the mud or sand and, to facilitate that burrowing, have become elongated; namely, the soft-shelled clam, *Mya arenaria*; the razor clam, *Ensis americana*, and *Solenomya velum*. This great group of bivalves represents then a society of animals that are fairly common because of favorable food conditions, but very helpless and much exposed to predaceous animals, were it not for their hard shell or their habit of burrowing into the mud.

b. *The crawling species* belong chiefly to the three groups of Mollusca, Echinodermata, and Crustacea. The crawling molluscs are slow-moving snails (Gastropoda) which are there partly to feed on decaying animal and vegetable matter, partly to feed on the growing *Ulva*, and partly to prey upon such living animals, chiefly bivalves, as have no means of escape. The chief omnivorous and carrion snail is the mud snail, *Nassa obsoleta* (Fig 4), which is abundant everywhere and even remains exposed on the middle beach at low tide, if busy feeding on a dead oyster. The little snails, *Anachis avara* and *Astyris lunata*, feed on the *Ulva*, or sea lettuce. The carnivorous species are of larger size and include two Muricidae, *Eupleura caudata* and *Urosalpinx cinerea* ("oyster-drill"), and the great whelks, *Fulgur caniculatum* and *Fulgur carica*. All these feed upon oysters, scallops, and other surface bivalves by drilling holes through the shell. Two species of Naticidae, *Neverita duplicata* and *Lunatia heros*, seek out the burrowing lamellibranchs, and so we find them burrowing into the sand. Then, too, they find in the sand of the beach the proper material for their egg cases, which are made out of agglutinated sand molded in the shape of a spiral collar. The crawling echinoderms are chiefly the starfishes, which are here because of the oysters and other bivalves upon which they prey. They cannot bore through the oyster's shell, and so they smother it until it is forced to open its valves for fresh water. The crawling Crustacea, finally, feed on organic debris of all sorts. Here belong the crabs, such as the three spider crabs, *Libinia canaliculata*, *Libinia emarginata*, and *Libinia dubia*, of which the latter comes farthest in-shore. Here, too, are the three mud crabs, *Panopeus depressus*, *Panopeus herbstii*, and *Panopeus sayi*, of which three the latter is found nearest the sand spit. On the very edge of the submerged zone are found also the two hermit crabs; the small one, *Eupagurus longicarpus*, finds protection for its abdomen in the cast-off shells of the small gastropods *Nassa* and *Anachis*. The large species, *Eupagurus pollicaris*, occupies such large shells as those of *Lunatia heros* and *Fulgur carica*. These scavengers, carrying their borrowed shells behind them, travel quickly along, but just below, the edge of the water, seeking for dead fish and other organic matter that may be resting there. Finally, the horseshoe crab, *Limulus*

polyphemus, the largest and most aberrant of our Crustacea, will be seen, especially during June, traveling over the shallow water and occasionally coming to land to lay its eggs in the sand.

c. *The burrowing animals* of the submerged zone constitute a remarkable fauna of, for the most part, elongated animals. We have already seen that many molluscs burrow. So do a few sea anemones, such as the white-armed sea anemone, *Sagartia leucolena*, and the flesh-colored or white *Halocampa producta*. These sea anemones seem to feed on bits of organic remains, of which the sand is full. The other burrowers are here for a similar purpose; the circumpolar sea cucumber, *Synapta inhærens*; the worm that shows affinities with vertebrates, *Balanoglossus Kowalevskii*; two nemertines, *Cerebratulus leidy* and *Cerebratulus lacteus*; and some seventeen different kinds of jointed worms, Annelida. All these find shelter, moisture, and food in the sand and mud beneath the sea bottom. But their immunity from attack is not complete, for as the moles have followed the earthworms and insect larvæ into the sub-aerial ground, so have several predaceous Crustacea followed the annelids into the mud. These are: the mantis shrimp, *Squilla empusa*, which is only a little smaller than the lobster, and *Callinassa stimpsoni* and *Gebia affinis*, that are somewhat smaller than a large crayfish.

d. *The swimming animals* are partly scavengers and partly predaceous. To the first class belong the prawn *Palæmonetes vulgaris*, which scours the edge of the tide for floating débris; and also the swimming crabs, the blue crab, *Callinectes hastatus*, and the commoner "lady crab," *Platyonichus ocellatus*. Here, too, we may place the little killifishes, *Fundulus*, although these pick up many live shore snails. The majority of the fishes are predaceous, feeding on the crawling and even the burrowing species that I have enumerated. The "skates" that lie close to the bottom catch burrowing worms and molluscs and also the snail *Lunatia heros*. The sand sharks and dogfish (*Carcharias littoralis* and *Mustelus canis*) gather in the spider crabs, squillas, and hermit crabs. The flounders, likewise, living close to the bottom, get *Gebia* and the prawns. The toad-fish, which lays its eggs in old shoes or in tin cans or under stones, feeds on the mud snail, *Nassa*, on crabs, and on prawns. Thus we see that in the shallow sea each species that occurs is present on account of particular relations that it bears to other species or to the non-living environment. The presence of the sharks is determined by that of the squillas, the squillas by the worms, the worms by the decaying vegetation, this by the living vegetation, and this by the salts and the nitrogenous food brought down by the creek from the valley above. This microcosm of the submerged zone affects in turn the lower and the upper beaches.

## II. THE FAUNA OF THE LOWER BEACH

As already stated, the lower beach is a zone where aquatic and terrestrial conditions alternate every day. On this account, and on account of the sand, it is an area devoid of all living vegetation excepting the unicellular algæ that grow upon the

stones (Fig. 4). It is also a region where oxygenation is combined with abundant moisture, so that conditions peculiarly favorable for respiration would seem to be afforded.

A fine layer of silt is dropped over the whole surface with each flood tide, affording thus abundant but microscopic food. But, on the other hand, it is a region of great exposure to terrestrial animals; so that only the stratum a little below the surface offers great safety. Also the lower beach is a region of wave action which makes it difficult for animals to secure a permanent place on it. Finally, and most important of all, the waves and currents cause the sands to shift, and this adds to the difficulty of maintaining a foothold. Consequently there are but few animals living on the lower beach, and such as there are live a curious and very strenuous life.

All over the lower beach will be found, upon careful examination, large numbers of extremely minute and active insects belonging to the group of Thysanura. These are arctic forms of Collembola of the species *Xenylla humicola*, O. Fabr. and *Isotoma besselsii*, Pack., together with an occasional *Anurida maritima*, Guer.<sup>1</sup> These Collembola are feeding on the rich microscopic débris which has been dropped on the lower beach. Being insects, they are air breathers; and the question arises: What do they do when the tide comes in? To answer this question I made measurements of the area occupied by the Collembola at different stages of tide. At tides lower than one-half tide the upper limit of the Collembolan zone is about nineteen meters north of the storm-cut bluff, or about ten meters north (*i. e.*, seaward) of the mean high-tide line. The lower limit is about two meters from the momentary tide-line. As the tide retreats the lower limit follows, while the upper limit remains constant. Thus, on September 10, 1901, with falling tide, the following determinations were made:

HOUR	DISTANCE FROM STORM BLUFF	
	Upper Limit of Collembola	Lower Limit of Collembola
3:10 P. M.....	17.7 m.	28.4 m.
3:30 P. M.....	17.7 m.	29.5 m.
4:50 P. M. (low-tide).....	17.7 m.	31.1 m.

Note that the lower limit of the Collembola travels down *pari passu* with the tide.

As the tide rises, the Collembola tend to retreat before the edge of the water, so that they are even crowded together there. Thus, on September 10, at 7 A. M. (one-third tide, rising), the lower edge of the Collembolan zone was about three meters away from the water's edge. As the tide rises still higher they crawl into the sand, until, at high tide, most of the Collembola are under the sea. But not all of the Collembola are there. At high tide one finds some of them floating on the quiet water out

<sup>1</sup>For the determination of these species I am indebted to my friend, Dr. J. W. Folsom, of the University of Illinois. (*Cf.* Wahlgren, 1899; Folsom, 1901.)

at a distance of ten meters or more, and moving hither and thither upon the surface. We conclude then that the rising tide has caught up with certain of the little insects. They rest upon the surface of the water by virtue of numberless fine hairs with which they are covered, in which the air is entangled so that the bodies of the insects are prevented from getting wet. These are chiefly Anurida.

A second species that occurs on the middle beach in great numbers during the latter part of June is the horseshoe crab, *Limulus polyphemus*. This occurs here because it lays its eggs in the sand of the beach, thereby reaping the advantage of the superior oxygenation afforded by this situation over sand constantly submerged. The eggs are laid in nests containing several hundred each. The eggs are oval and about two millimeters in diameter. Each is enveloped in a tough membrane so that the sand cannot injure it. The position of the nest may be detected by a slight depression in the surface over it, and by the absence of pebbles. Not all the middle beach is occupied by these nests, but only those regions where the sand is coarse enough to let water through readily, but not so gravelly as to make hard digging. The east end of the sand spit where the current flows swiftest affords the best conditions, and here the nests are crowded together. In June also one finds many carcasses of female horseshoe crabs that have died in consequence of oviposition; for, as in many other species, oviposition is accompanied by a great mortality. Most of these carcasses are eventually thrown up to the high-tide line, and their fate will be considered in connection with the fauna of the upper beach.

Finally, mention should be made of the great annelid, *Nereis limbata*, that occurs burrowing in the sand above low-water mark. This again is confined to the tip of the sand spit where oxygenation is best carried on.

### III. THE FAUNA OF THE UPPER BEACH

The upper beach I shall define as the zone including the high-tide line and above to the storm bluff (Fig. 3 left, Fig. 5). This region is inhabited by a very few annual plants; its main characteristic, however, is the débris cast up by the sea (Fig. 5). All over the world the upper beach is the graveyard of the shallow sea. In this graveyard two sorts of remains are found: first, such as have been floating on the surface of the sea; and, second, such as have fallen to or were lying on the bottom of the shallow sea. The floating remains are carried in toward the shore by winds from the sea. If the sea is quiet, they are merely dropped at the time the tide begins to fall; consequently they mark the high-water line (Fig. 5). If the sea is heavy, the floating or drowned débris may be thrown against the upper part of the upper beach and even against the storm bluff. This flotsam and jetsam consists, in the first place, of such things as lumber and articles of wood and cork; fruits and seeds; bits of eel grass; stems of last year's marsh grass, *Spartina*; fronds of *Ulva* torn from the mud flats; jelly fishes; drowned insects, especially heavy-bodied beetles, which have probably been blown out to sea and been drowned or have fallen in during migration. The

second class includes chiefly empty shells, whose inhabitants have perhaps met with a violent death through predaceous animals, or the smothering of a stirred up muddy bottom,<sup>2</sup> also the dead bodies of Crustacea, such as *Limulus*. This débris is piled up at the lower edge of the middle beach and is renewed twice a day. Especially, however, after a storm is the accumulation large. At such times and at certain seasons of the year one may meet with particular species in large numbers. Thus, early in September, 1901, as the young *Pectens* were swimming into the inner harbor, a combina-



FIG. 5.—Photograph of north side of sand spit, near the western end, at low tide. In the central foreground is the high-tide line, marked by a mass of débris. On the left is the gravelly lower beach; the middle beach and storm bluff are at the right.

tion of high tide and sea breeze left thousands of them stranded on the upper and even on the lower beach.<sup>3</sup> The drowned insects are largely leaf eaters (*Chrysomelidæ*, including the Colorado potato beetle) and, especially in the early summer, ladybirds (*Coccinellidæ*) of various species. All of these constitute a rich, frequently replenished food supply, the only disadvantage connected with it being the dangerous proximity of the sea, with its occasional very high tides and its storm-born breakers. As could have been anticipated, certain animals have come together to make use of this food material. Some are herbivorous, others are scavengers, and others still are predaceous.

<sup>2</sup> In March, 1890, the levee gave way on the left bank of the Mississippi river above New Orleans. The waters pouring through Lake Ponchartrain into western Mississippi Sound so stirred up the muddy bottom that the great beds of oysters of this region were killed. (Smith, 1894.)

<sup>3</sup> During a visit to Santa Rosa island, outside of Pensacola Bay, Florida, in March, 1902, I found the beach covered with thousands of Portuguese men-of-war (*Physalia*), and the floating gastropod, *Janthina fragilis*, thrown up by the southerly storms.

Of the herbivorous feeders on the wreckage of the sea may be mentioned, first of all, the Amphipoda, marine Crustacea which are so adapted to a terrestrial life that they are rarely submerged. At Cold Spring Harbor two species are found—the small, dark *Orchestia agilis* and the large, sand-colored *Talorchestia longicornis*. Both may be seen in great numbers by turning over some of the cast-up *Ulva* fronds, under which they live and upon which they feed. Here they dwell in a saturated atmosphere and so need no special modification of the respiratory apparatus to fit them for breathing air. They both burrow, also, forming holes varying from three to five millimeters in diameter in the fine sand under or slightly above the line of wreckage. These holes enable the amphipods to reach moisture, they prevent them from being swept away by the sea, and they may serve as nests for eggs. For some reason the *Talorchestia* only is found at the tip of the spit. If it be asked why these Amphipods have left the water thus to assume a half-terrestrial life, I think it is a sufficient answer to say, first, that they find here abundant food; second, that they are here comparatively immune from the attacks of their greatest enemies—the fish; and, third, that their organization permits them readily to assume a semi-terrestrial life, as is shown by the fact that some of their allies have become even more terrestrial than they. (Compare *Talitrus platycheles*, and *Talitrus saltator*, Semper, 1881, p. 188.) That the *Talorchestia* is no longer an aquatic animal is shown by the way it retreats before the tide, especially if abnormally high.

Secondly, rove-beetles (Staphylinidæ) of the genus *Bledius* are found in the débris. This terrestrial insect is here found side by side with the marine *Talorchestia*, even burrowing into the sand. It feeds upon decaying vegetable matter. A third organism found under the débris is a minute white earthworm of world-wide distribution. This is *Enchytræus albidus* Henle (*Halodrilus littoralis* of Verrill, 1873).

As the plant débris is being devoured by the amphipods, staphylinids, and *Enchytræus*, so the animal remains are being carried off by a number of scavengers. Among these the ants are the most important; there are two species of them. The first, *Formica rufa* var. *obscuriventris* Mayr, is reddish brown and about four millimeters long (see Emery, 1893). It digs holes in the sand in the upper part of the upper beach, the grains of sand being brought individually to the surface and deposited in a ring around the hole. This ant also occurs under the shelter of boards and logs. Immediately after the tide has begun to fall and dropped its burden of carcasses these ants sally forth in paths that run perpendicularly to the high-tide line and begin to seize and carry to their nests the drowned insects that have been left there stranded. A second species of ant has its home somewhat farther out of reach of the tide; but its habits are quite similar.

There is, however, a larger carrion fauna to be utilized. I have already referred to the dead horseshoe crabs and the dead molluscs that are left on the shore. These soon attract great numbers of the flesh flies (*Sarcophaga carnaria*) which lay their eggs in the carrion. A second fly with bronze abdomen (undetermined) is also found

here. To test the quickness with which these flies accumulate I took a recently drowned turtle from the water at 3 P. M. July 7, and left it at the edge of the retreating tide. At 4:15 P. M. (wind south) I counted over thirty of the bronze flies upon it. It remains to be determined whether it is chiefly sight or smell that attracts them.

Underneath the carcasses of the horseshoe crabs one will find also large numbers of the carrion beetle, *Necrophorus*, and larvæ of the museum pests (*Dermestidæ*).

The rich fauna of carrion feeders and herbivorous and omnivorous species determines still another fauna, namely, a predaceous one. Thus, running spiders, almost as white as the sand, frequent the upper beach and sometimes pass down to the upper part of the lower beach. These belong to the species *Lycosa cinerea*—a species that occurs commonly in Europe also, and is by no means confined to the sand of *beaches*. I have seen them carrying off *Orchestia* toward the storm bluff. More powerful still are the robber flies (*Asilidæ*) which pounce upon carrion flies. Their path of flight is curved, and at the moment of alighting an offset is taken several centimeters to one side. Altogether the irregular flight seems well adapted to the end of putting the victim off its guard, like the curve in the path of the ball thrown by an expert baseball player. Tiger beetles also occur abundantly on the beach in the bright sunlight, especially in August and September. They are chiefly of the species *Cicindela repanda* Dej. They are the most rapid fliers among beetles and feed upon the other insects of the beach. Finally, all these insects must fall victim to the swift and powerful swallows which course up and down the beach, especially in the latter part of the afternoon.

On the upper part of the upper beach there occur also a few stragglers from the vegetation-grown top of the sand spit. Here one finds grasshoppers almost as white as the sand. The white color of this species seems partly determined by the color of the background, for placed in a cage on grass these spiders become darker, as experiments at the Biological Laboratory have repeatedly shown. Whether the grasshoppers feed upon the dead vegetation of the tide-line was not ascertained. Crickets, *Gryllus abbreviatus*, quite as black as those living in the grass, occur scatteringly on the upper beach; and under the waste lumber the sow-bug, *Oniscus*, which is only half adjusted to a terrestrial existence, finds a living on the water-soaked wood.

#### B. THE BEACH ON THE TIP OF THE SAND SPIT

The tip of the spit is transitional between the inner and the outer sides. Here the sandy beaches of the north side gradually pass over into the mud-flats of the south side with their thick growth of marsh grass, *Spartina*. Indeed, at the tip of the spit there is a constant struggle going on between the upbuilding tendencies of the *Spartina*, which tends not only to hold the mud in which it grows together, but also to accumulate additional silt, and the scouring away tendencies of the tide that rushes through the gut. Wherever a weak spot appears in the mass of *Spartina* there a channel becomes gradually worn through (Fig. 6). These channels gradually widen and may anastomose, and thus the *Spartina* be left on elevated hummocks, which would



seem destined to become smaller and smaller until entirely washed away. But from this fate they are preserved by an interesting association. The current that rushes through the channels carries with it an abundant supply of microscopic food, such as lamellibranchs can make use of. This food is taken advantage of by the mussels which come to line the muddy banks on the channels, and form so close a wall that erosion is almost completely stopped (Fig. 6). Thus the mussels assist the *Spartina* in their constructive work. The mussels that line the banks are *Modiola plicatula*, *Modiola*



FIG. 6.—Photograph taken at the inside of the hook of the sand spit, looking north, at low tide, through one of the passages scoured out by the tidal currents. At the base of the *Spartina* patches, on the right, are seen some of the beds of mussels which protect the roots from exposure.

*modiolus*, and *Mytilus edulis*, the first-named being the most abundant. The channels have irregular bottoms in which shallow pools of water stand when the tide is out. Here the mud snails, *Nassa obsoleta*, aggregate, scarcely submerged. High up on the stems of the *Spartinas*, exposed to the air during perhaps half the day, are found clinging the *Littorinas*, whose lack of a siphon makes it necessary for them to keep out of the mud. *Littorina rudis* and *Littorina palliata* were still the prevailing species in 1898 (see Balch, 1899), but in 1901 *Littorina littorea*, which is rapidly advancing up the harbor, had gained a marked predominance.<sup>4</sup> The independence of the sea water that is exhibited at the tip of the sand spit is also illustrated in the marsh at the head of the harbor through which Cold Spring creek runs. On this marsh *Littorina* occurs

<sup>4</sup>This habit of clinging to rushes is even more exaggerated in the southern *Littorina irrorata*. For, in a visit to the Lagoon on Ship Island, Mississippi Sound, in March, 1902, I found nearly all of them living on the stems of the

short marsh grass twenty to thirty centimeters above the water level and exposed to the sunlight. Cooke (1895, pp. 20, 93, 151, note) cites other cases of *Littorina* living out of water.

at places where it is submerged for only a short time at high tide and then under water that is nearly fresh. *Littorina rudis* behaves similarly in other parts of the world. Thus Fischer (1887, p. 182) states that at Trouville (Calvados) on the English Channel he has found *L. rudis* on rocks two meters above the other marine animals and moistened only by the highest tides. In fact, according to Simroth (1891, p. 84),



FIG. 7.—Photograph taken at half tide from the base of the sand spit, looking east, showing inner harbor, the hook of the spit, and the gut beyond.

species of *Littorina* pass the winter out of water with their gill chambers full of air. *Littorina rudis*, then, has evidently progressed far on the road toward adaptation to a terrestrial life—a road that the Pulmonata must have traveled long ago.

#### C. THE INNER EDGE OF THE SAND SPIT

Along the whole length of the inner beach, not far from the high-tide line, occur the holes of the fiddler crabs. These crabs belong to two species, namely, *Gelasimus pugnax* and *Gelasimus pugilator*. A remarkable thing in the distribution of these species is the fact that although their habitats are not markedly different their areas of distribution are so well defined that they hardly overlap. Both species occur at the edge of the *Spartina*. The *pugnax* is found all the way from the western, proximal end of

the sand spit to about two-thirds of the way toward its eastern point. Then pugilator abruptly comes in. For a distance of a meter or so the two species occupy ground in common, and, so far as I could make out, peacefully. The pugnax alone occurs at the head of the inner harbor. It burrows in the banks at a level that is reached only by the high tides. Walking along the beach at high tide July 7, I found that many of the fiddlers had migrated to above the high-tide level. It is clear, I think, that they do not find submergence altogether agreeable, and it is probable that prolonged submergence would drown them as it does *Ocypoda arenaria*, the sand crab of the beaches south of Cape May. *G. pugnax* prefers the marshier ground and the higher water, and it is probably that preference which determines its spacial separation from pugilator on the sand spit.

#### D. THE TOP OF THE SAND SPIT (TERRESTRIAL BORDER ZONE)

Above the storm bluff on the outer beach, and at a less well-defined line on the inner beach, lies the zone of permanent vegetation in which certain shrubs have gained a foothold. Here the fauna at once assumes a strictly terrestrial aspect. No close ally, even, of a marine form occurs. On the contrary, the animals living on vegetation are precisely those species that occur in the fields, especially the plant feeders: the plant lice (*Aphidæ*), the leaf beetles (*Chrysomelidæ*), the bright-colored *Buprestidæ*, and the various blister beetles. On the sandy ground are sand-colored grasshoppers, sand-colored spiders, *Lycosa cinerea*, and also small black spiders (*Lycosa communis*; cf. Emerton, 1885), black crickets, and little red ants apparently identical with species that people the upper beach. Over the vegetation wandered, in early July, an abundance of the predaceous dragon-flies, a black wasp (*Polistes*), and an occasional dusk-flying butterfly (*Hesperidæ*)—quite the fauna of a meadow not far from water.

#### SUMMARY ON THE ANIMAL ECOLOGY OF COLD SPRING BEACH

The outer beach is a region of breakers where débris is thrown on the shore. The submerged zone is crowded with marine animals, some of which make their way out of the water and others of which contribute the débris with which the upper beach is strewn. The lower beach is covered with *Collembola* that feed upon microscopic organic débris and crawl into the sand at high tide. The line of débris is a rich feeding-ground for animals that live on vegetable matter and on carrion. The débris feeders—*Amphipoda*, *staphylinids*, earthworms, ants, carrion flies, necrophorous beetles, attract a predaceous fauna of spiders, robber flies, and tiger beetles. These predaceous species are fed upon, in turn, by the swallows.

The tip of the beach, where the marsh grass grows, is a region of swift currents which the lamellibranchs (mussels) find advantageous because of the food that the currents bring. The currents tend to wear away the spit, but the mussels grow so abundantly on the banks of the channels as in turn to protect these banks from further erosion.

The inside of the sand spit is a region of sedimentation. Plants grow here, and here the plant-feeding snails and fiddlers live. The organisms that are found on the beach are not accidentally there, nor is the fauna determined by causes remote and too complex to be unraveled. The fauna is determined by definite proximate causes of a simple sort that act, the world over, in the same way, and so give to a similar sea beach in other parts of the world a similar collection of animals—excepting that each species may be replaced by another.

#### COMPARISON OF COLD SPRING BEACH WITH THAT OF LAKE MICHIGAN NEAR CHICAGO

The question arises: How far is the fauna of the sea beach determined by the beach conditions of sand, sunlight, and proximity to a body of water with its strand zone of débris? Will beaches in general, whether of a fresh-water lake or of the sea, tend to have the same fauna? To test this question I have examined the fauna of the shore line of Lake Michigan, south of Jackson Park, Chicago. Here one finds a sandy beach essentially like that at Cold Spring Harbor. On one side extends a huge body of water which differs from that of the harbor chiefly in its lower specific gravity and in the absence of marked tides, but resembles it in its waves. We may recognize here a submerged beach and a terrestrial beach.

##### I. FAUNA OF THE SUBMERGED ZONE

This inundated part of the beach supports, as one would expect, a fauna the species of which are quite unlike those of the sea. Yet we may recognize a sessile fauna, a crawling fauna, and a swimming fauna.

a. *The sessile fauna* includes here, as in the sea, the lamellibranchs. These belong to two families, the Unionidæ and the Spheridæ. These, like their marine allies, feed on minute organic particles, chiefly algæ. The Unionidæ are the large forms and seem to take the place of the marine *Macra*, *Venus*, and *Mya*. They occur in the streams and lakes of all parts of the northern hemisphere, but the group is best developed in North America. The Spheridæ are small, and take the place of the *Nuculas* of Cold Spring Harbor and *Donax* of our southern seashore. They are found in the streams and lakes of all countries.

b. *Crawling animals* belong chiefly to the groups of gastropod Mollusca and Crustacea—the Echinoderma being wholly absent. The snails are mostly small and seem to replace the *Littorinas* and the *Rissoas* of the seacoast. The principal crawling crustacean is the isopod *Asellus communis*, which lives on the wood and among the roots of the shore line. The group is, indeed, poorly represented here as compared with the sea. Burrowing animals seem to be almost entirely absent, possibly on account of the absence of such predaceous forms as occur in the sea.

c. *The swimming animals* are here, as in the sea, partly scavengers and partly predaceous. First of all we have in the water above the shore numerous Entomos-

traca. The prawns of the sea are replaced by a very closely related species, *Palæmonetes exilipes*, which may have gained the great lakes by the way of the Mississippi river, in which it is abundant. The marine lobster is replaced by the crayfishes (*Cambarus propinquus* and *C. virilis*). The predaceous forms are the fishes which feed largely upon the snails and the Crustacea.

## II. FAUNA OF THE BEACH

In the tideless lake the lower and upper beaches are hardly to be distinguished. On the lake strand *Collembola* are found just as on the lower sea beach. In the line of débris that the waves deposit are found the wrecks of all the shallow-water forms of which I have spoken, and, in addition, the carcasses of vast numbers of insects that have fallen into the lake, have drowned, and are cast up by the waves. This wreckage line affords, then, just the feeding-ground for inland species that the marine species find on the coast. What animals do we find here? The burrowing *Orchestidæ* seem, indeed, to be absent, but there is a closely related species (*Allorchestes*) that lives in the shallow water. That it is not a beach burrower may be due to just these causes that have eliminated the burrowing habit in general. But under the débris rove-beetles and insect larvæ are to be found feeding on the vegetable matter. And small red ants build nests on the beach and visit the débris for the carcasses of insects. A similar carrion fly and carrion beetle (*Necrophorus*) occur. Feeding upon this fauna is a running spider (*Lycosa cinerea*) the same as that of the coast. Here, too, occur robber flies and tiger beetles, and even white grasshoppers. Thus the lake beach, having a similar strand zone of decaying vegetation and plant wreckage with the sea, has, at a distance of nine hundred miles from the sea, excepting certain strictly marine species, practically the same fauna as the sea. The conclusion to be drawn from this fact is the immense importance of habitat (*i. e.*, of environmental details) in determining similarity of fauna, or, in other words, the fauna of a point is, within limits, determined rather by the environmental conditions than by the geographical position of the point.

## REMARKS ON THE THEORY OF ADAPTATION

Everyone must admit the fact of adaptation of the structures of animals to their environment. The generally accepted theory to account for this is that of Darwin and Wallace that a species coming into a new habitat gradually acquires a fitness to that habitat by the killing off of the less fit individuals born into the species. There are some cases, as, for instance, that of the leaf insects, that of the fungus beetle, and those of mimicry, that I can see no other explanation for but this, that an external condition existed first and a structure or coloration was acquired by the race that fitted or adjusted it to that external condition.

We must not, in accepting any theory as a true one, try to force it as a universal theory and become blinded to other possible theories. Now there is another and funda-

mentally different possible theory of adaptation, and this is that the structure existed first and a fitting environment was sought or fallen into by the species having the peculiar bodily condition. Thus the adaptive result is, on this theory, not due to a selection of structure fitting a given environment, but, on the contrary, a selection of an environment fitting a given structure. I shall now consider some special cases that are best explained on this theory. Thus, Eigenmann (1899) shows that the cave fishes, which in many points show an adaptation to the cave environment, are not to be thought of as having accidentally got into caves and as having subsequently gained a structure fitting them for that environment. But, on the contrary, as they all belong to one family, their getting into the caves was evidently not an accident. Moreover, this family includes species that are structurally especially fitted for cave life, even when they occur in regions where there are no caves and never have been any. They shun the light, and live in crevices and under stones. Their bodily conditions fit them for cave life and when, in their constant search for dark holes, some of them succeeded in getting into caves, they naturally thrived there.

Again, in many cases of parasitism among snails the radula is known to be absent altogether, and this has been accounted for by Cooke (1895) on the hypothesis that these snails lost their teeth through disuse. However, it is pointed out as a curious fact that the same absence of a radula occurs in species of *Eulima* known to be not parasitic. Cooke suggests the hypothesis that in cases like this the form must have derived from parasitic ancestors. It is equally probable that *Eulima* is a mollusc that will probably soon be driven to parasitism because it has no radula.

Now, that which is true for the cave animals, and probably true for edentulate snails, is illustrated time and time again in the animals of the beaches. We have seen that the Anurida are covered with fine hairs, which enable them to float upon the tide and thus keep them from drowning. Are not the fine hairs a remarkable adaptation to the necessities of the situation? They certainly are, but the probability is that the hairs were not developed to meet this situation at all; at least, such a coating of fine hairs is widespread among Collembola, and the hairs subserve a variety of functions. Thus, Schaffer (1898) finds that the long hairs protect the animal against the action of the sun's rays in the case of certain species that live on leaves; and the importance of such protection would seem to be great, for Absolon (1900) finds that certain cave forms of Collembola, which, so far as he describes them, seem to be scantily covered with hairs, are killed by a few minutes of exposure to sunlight. Hairs are, we may then say, common occurrences on the thin-skinned Collembola. The hairs are important to keep the thin skin from desiccation. Because the skin is thin, the Collembola favor damp or wet places; just because they are covered with hairs, they can float on the water; just because they can float on the water, they can live on the lower beach. Also, they find here their appropriate food. Having by some means got to the beach, they remain there, because they find the conditions existing on the beach peculiarly suitable.

This law is illustrated again by an inhabitant of the upper beach, *Oniscus*. *Oniscus* seems, on the whole, rather poorly adjusted to a terrestrial life. Its gills lack the well-developed tracheal chambers of the wood louse, *Porcellio*, and the pill bug, *Armadillidium*, its close relatives (Stoller, 1899). Correspondingly we find it only in moist situations, under logs, in cellars, in greenhouses, etc. On the other hand, *Porcellio ratzburghii*, Brandt, is a species in which all five pairs of outer gills possess tracheal chambers. As Stoller (1899) remarks: "This species lives in situations where the air is charged with moisture only in a moderate degree in excess of that of ordinary atmospheric air. Their habitat is under the bark of dead trees, and they may often be found a meter or more above the ground." Now, experiments that I have made show that a water-inhabiting isopod (*Asellus*), if taken out of the water, will go back into it if free to do so; and *Porcellio*, if put in water, will leave it for dry land, if free to do so. Similarly we may conclude that *Oniscus* chooses a situation that affords the requisite moisture. Shall we conclude that the reason why *Oniscus* has no tracheal chambers is the result of its living in a moist situation, or is it the cause? We find it where it is because it is hydrotactic. Now, is it hydrotactic because it has no lungs, or is it without lungs because it is hydrotactic? Certainly it would be rash to assert the latter, even though we cannot prove the former.

So likewise we find *Nassa*, which has a siphon to enable it to draw pure water from above the mud, living in the mud; whereas *Littorina*, which has no such siphon, clings to the stems of the marsh grass above the mud. Can we say that *Littorina* has no siphon because it clings to the marsh grass, or does it cling to the marsh grass because it has no siphon? I maintain that the latter is no less true than the former.

Let us consider still one other case. We have seen that the mussels cling to the banks of the channels in such numbers as to make a protecting wall. Of the advantageousness of that situation for the mussel as a lamellibranch there can be no doubt; abundant food and oxygen are brought to its doors every day. The wonder is that no other lamellibranchs than the mussel occupy this favorable situation. Why is this? It is because the mussels are the only species living about the sand spit that have a byssus in the adult stage. Lacking a byssus, the other species cannot attach themselves to the banks. Now, does *Mytilus* have a byssus because it tends to attach itself to banks, or, being provided with a byssus, was it led to take advantage of the favorable position offered? Did the situation or the organ precede? In this case we may see, I think, the necessity of the organ being well developed before the special habit (of attachment) could be exercised. However, it is quite likely that the byssus has been improved in the race by selection, and with every step of improvement the race has been able to take up a more and more advantageous habitat.

This brings us, indeed, to the most reasonable hypothesis of adaptation, namely, the combination of the improvement of the organ to meet the requirements of the environment, through selection, and of improvement of situation to meet the abilities of the organism. There have gone on, hand in hand, a selection of more appropriate

organs and of more congenial habitats. Adaptation of organization to environment has been effected by the double process of selection by environment of the most appropriate organization and by the organism of the most congenial environment.

This hypothesis, I think, should be welcomed by those palæontologists who, like Osborn (1897), are led from their phylogenetic studies to conclude "that there are fundamental predispositions to vary in certain directions." They help out, too, I believe, the fundamentally important observations and experiment of DeVries (1901), who finds that race change is a series of steps, of mutations, that may often have no relation to adaptation; the adaptation comes later. For all those theories, in general, that assume that change of specific structure occurs independently of selection of the fittest, the hypothesis here proposed must be considered a welcome complement. It may be well to point out that the selection of a fitting environment is not confined to migratory animals. It is applicable to all organisms that have a means of dispersal. The seed that falls upon good ground—the race that gets into a favorable environment—will survive.

The theory may thus be summarized: The world contains numberless kinds of habitats, or environmental complexes, capable of supporting organisms. The number of kinds of organisms is very great; each lives in a habitat consonant with its structure. Each species is being widely dispersed, and, by chance, some members of a species get into an environment worse fitted for them; others into one better fitted. Those that get into the worse environment cannot compete with the species already present; those that get into a habitat that completely accords with their organization will probably thrive and may make room for themselves, even as the English sparrow has made room for itself in this country. This process may go on until the species is found only in the environment or environments suited to its organization. As Darwinism is called the theory of the survival of the fittest organisms, so this may be called the theory of segregation in the fittest environment.

In conclusion I repeat that the theory of segregation in the fittest environment does not replace that of survival of the fittest organism, but is complementary to it. It has this *raison d'être* that it shows how unadaptive mutations may become adaptive *if only they can find their proper place in nature.*

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**THE FINER STRUCTURE OF THE NEURONES IN  
THE NERVOUS SYSTEM OF THE WHITE RAT**



# THE FINER STRUCTURE OF THE NEURONES IN THE NERVOUS SYSTEM OF THE WHITE RAT

SHINKISHI HATAI

THE two problems which we have to consider are the fundamental structure of the ground-substance of the nerve-cells and the manner in which the axone of one neurone terminates on the dendrites or on the cell-body of another. Many opinions and theories concerning this structure and these relations have been put forth during the last decade, and as a consequence the literature of this subject is already very large. Nevertheless it cannot be said that either of the questions has been definitively solved. With the aid of a new technique we have been able to see some structures and relations not heretofore described, and it is the object of the present paper to interpret and depict these new appearances.

## I. TECHNIQUE

For the present investigation a large number of white rats having body-weights ranging from 5.4 to 185 grams were employed. The material was preserved with Gilson's fluid, Carnoy's mixture, and the mixtures devised by the present writer (1901); as the staining agents, saturated aqueous solution of thionin followed by a 1 per cent aqueous solution of erythrosin; Heidenhain's iron-haematoxylin, and Ehrlich's triacid method were used. In applying these several methods the author's directions were strictly followed. In each case, after imbedding in paraffin according to the usual procedure, sections were cut three micra in thickness.

Besides these methods, the present writer tried another method which gave very satisfactory results in the study of the ground substance of the nerve-cells as well as in that of the finer structure of their processes. This method is as follows: As soon as the fresh material was removed from the body, it was put directly into the following solution. The pieces should not be more than 5 mm.<sup>3</sup>.

1. Acetic-picric-formalin mixture (after the writer, 1901) - - - 250 c.c.
2. Acid fuchsin, saturated aqueous solution - - - - - 50 c.c.

After twenty-four hours the pieces were taken from the solution and were teased in glycerin with fine needles. In this teased preparation only the achromatic substance of the nerve-cells and processes stain, while the rest of the cell-substance remains unstained. For this reason it offers us several advantages for the study of the minute structure of the axone and dendrites not possessed by other methods which were employed. Although this preparation gives such satisfactory results, it cannot be preserved permanently, as the color soon fades. To meet this difficulty, the material thus preserved and stained may be imbedded in paraffin after having been carried

through 95 per cent. and 100 per cent. alcohol and xylol. In this case, however, the section must be restrained upon the slide. These restrained preparations give clearer pictures than those obtained by other methods, and, in addition, show a distinction between the axone and the dendrites. The appearances about to be described were obtained by this method.

## II. THE FINER STRUCTURE OF THE GROUND SUBSTANCE OF THE NERVE-CELLS, ESPECIALLY OF THE SPINAL GANGLION CELLS

The spinal ganglion cells in the white rat present at least two varieties differently characterized; one of the varieties is larger in size and stains faintly with eosin or erythrosin; while the other shows just the opposite characters, that is, the cell-bodies are smaller and stain deeply with these solutions. The present description of the spinal ganglion cells is based on the study of the larger variety which has been regarded by the writer as the fully matured form (1901).

In general, the cell-body shows a circular or a somewhat oblong shape, containing a single nucleus which lies at or near the center of the cell. The nucleus contains a large number of the oxyphile particles of various sizes. These particles are most abundant at the periphery of the nucleus as well as around the nucleolus which lies near its center. These particles hang along the fine filaments of the linin substance which forms a very complicated network in the nucleus (Plate XIII, Fig. 1). By using toluidin blue and eosin, the oxyphile substance and the linin stain red, while the nucleolus stains an intense blue, owing to the accumulation of the basophile substance around the oxyphile substance which forms the nucleolus proper (1901<sup>3</sup>).

The distinction between the oxyphile and the linin substance may be made out by the fact that the latter stains more faintly. The nuclear membrane is distinctly visible, showing a somewhat reddish brown color. The cell-body proper is composed of at least two different substances, as is the case in all other nerve cell-bodies, namely (1) the stainable substance known also as tigroid, Nissl's bodies, chromatophile particles, etc., and (2) non-stainable or ground substance. The stainable substance just mentioned appears to fill up the cell-body, except in two regions, one along the periphery of the cell-body and the other around the nucleus, the regions being known respectively as the peripheral and the inner, clear zones (Lenhossék, 1895). In these two regions the non-stainable substance alone is visible.

Under a low magnification the cell-body appears as a red homogeneous mass with a bluish tinge in it. A higher magnification of such a cell reveals a large number of minute meshes presenting a reticular arrangement. These meshes, however, are not similar either in shape or in size, but differ very widely according to regions in which they occur. They are formed by very delicate protoplasmic filaments within which minute granules are clearly distinguishable. These granules are known as "neurosomes." The neurosome seems to be a very highly differentiated cytomicrosome and to form the main part of the filament. The neurosome stains much deeper with eosin or erythrosin than ordinary cyto-microsome, and also is much larger than it.

As was pointed out by Held (1897), the neurosome is not only found imbedded within the filament, but appears also in the meshes between the filaments. The neurosomes in the region of the terminal end of the axis cylinder are very much larger than those found in the rest of the neurone (Plate XIII, Fig. 1).

As has already been mentioned, the meshes formed by the filaments are highly variable both in size and in shape. Generally, in the clear zone at the periphery of the cell-body, the meshes are larger and more conspicuous than in the remaining part. In the neighborhood of the axone hillock the meshes are not only much diminished in size, but they are also elongated. Around the nucleus these meshes reach a minimum size. The form of the reticulum at the periphery shows meshes of a somewhat polygonal shape, but in the remaining part of the cell these meshes are elongated, especially around the nucleus and near the axone hillock. These modified meshes present a fibrillar appearance, especially those around the nucleus as well as in the neighborhood of the axone hillock, owing to the alteration which has been described; that is, the elongation of the meshes diminishes the original space contained between the filaments and renders the filaments approximately parallel. In some cases several of these filaments unite together and form very thick strands. These secondary alterations take place throughout the cell-body and around the nucleus, but never occur at the periphery of the cell. Thus, the fibrillar structures as well as the fibrillar network within the cell-body are produced. These fibrils, therefore, are very different from those described by Apàthy and Bethe. According to Golgi (1898) and others, a modified silver-nitrate technique brings out a new structure within the nerve-cells. This structure presents very complicated network around the nucleus, and to this the name "endocellular network" has been given. It seems probable that the endocellular network just mentioned may be one expression of the elongated meshes of the fibrillar substance observed by the writer. Since Golgi's technique does not bring out the very minute structures, the figures obtained by him are only a fragment of the network which we have described. I will take up this point later on and will present the evidence I have for identifying this endocellular network with the structure to be seen within rats' nerve-cells.

Among the neurologists, two different views concerning the structure of the ground substance in the nerve-cells are held: These may be designated as (1) the fibrillar, and (2) the non-fibrillar or reticular. These two appearances in the protoplasm have been brought out by using different techniques. Apàthy (1895) demonstrated the fibrillar structure in the annelid nerve-cells by using gold chloride; Bethe (1897) in the crustacea, killed with nitric acid and stained with toluidin blue; Cox (1898) by osmic acid; Flemming (1895) by his own fixing agent; Dogiel by methylen blue; Becker (1895) by Weigerts' copper and hæmatoxylin stain; Kronthal (1895) by staining freshly crushed and dried specimens with methylen blue, etc., while the reticular structure was obtained by Bütchli, Held, Lenhossék, Van Gehuchten, Cajal, and others, using either strong alcohol, corrosive sublimate, Gilson's mixture, or Carnoy's solution.

The writer has had the opportunity to study the preparations made after the method of Bethe, Dogiel, and Kronthal, and to compare those with his own preparations from the white rat. In these cases, however, the writer was unable to see any fibrillar structure, such as had been described by those writers, but observed only a reticulum producing pseudo-fibrils. Although the reticular structure of the ground substance seems to be characteristic for young and unmodified nerve-cells, nevertheless in the large multipolar cells it has been altered to such an extent as to present a fibrillar appearance such as is seen in axone hillock of the spinal ganglion cells and around the nucleus. This alteration is probably due to growth changes, as was pointed out by the writer (1901<sup>4</sup>) in an earlier paper.

So far as my observations go, the fibrillar structure of the ground substance in the nerve-cells of the white rat is merely a modified network, and consequently it cannot be compared with the fibrillar structures described by Bethe and Apáthy.

### III. FINER STRUCTURE OF THE AXONES AND DENDRITES

Both the axones and dendrites are direct prolongations of the cell-body and present wide variations in their shape, size, and length, according to the cell-bodies from which they arise.

1. *Structure of the axones.*—An axone originates, as a rule, from a specially differentiated portion of the cell-body known as the “axone hillock.” The axone hillock appears under the microscope as a cone, being clearly marked off from the surrounding cytoplasm by the absence from it of the Nissl granules. Under a higher magnification this area of the axone hillock is seen to be composed entirely of delicate filaments formed by rows of neurosomes and stains more intensely than the rest of the cell (Plate XIII, Fig. 1). These delicate filaments run convergently from the cell-body to the axone and produce well-known radial arrangement of the filaments. These filaments, however, are not real fibrils, but, as has already been mentioned, they are modifications of the reticulum, and the so-called fibrils in this region are connected with one another by the delicate side branches. In other words, the axone, like the cell-body, is composed of a reticular arrangement of the cytoplasm and may be regarded as an extension of the cell-body proper. The ground substance, or the reticulum, of the cell-body, as well as the axone, is composed of cyto-microsomes and neurosomes. The neurosomes in the axone seem to be more differentiated than those in the cell-body proper, for they show a stronger affinity for acid dyes, especially in the terminals of the axones. It is interesting to note that the pseudo-fibrils in axones are packed very densely, and therefore the real structure of the primitive reticulum is hard to make out. The structure of the axone may be well studied by examining the cross-sections of the terminals (Plate XIII, Figs. 2, 3, 4; Plate XIV, Figs. 5, 6.) The neurosomes which form the terminals of the axis cylinder are very conspicuous, both by their size and by the manner in which they stain. The size of the individual neurosome in such terminals is a trifle larger than in the axone proper and stains a more intense red. It is already known that the

axis cylinder at its end enlarges greatly and forms the so-called "axis cylinder plate." An enlargement of the axone terminal may be seen in Figs. 5 and 6. Especially in Fig. 5, where the nerve-fibers enter into the granular layer of the cerebellar cortex, there is to be seen an enormous enlargement of the axones to several times their original diameters. A detailed description of the structure of the axone terminals and their relation to the surrounding neurones will be given later.

2. *Structure of the dendrites.*—The internal structure of the dendrites shows a close resemblance to that of the cell-body. Besides the ground substance which stains faint red, as in the case of the cell-body proper, it contains Nissl granules. Unlike the axone the dendrite contains but a small amount of the ground substance, and, further, the size of the individual neurosomes is approximately the same as that of the cyto-microsomes, where they stain more faintly than the neurosomes in the axone. In other words, the neurosomes in the dendrites do not show much differentiation from the cyto-microsomes. The reticulum, however, presents a marked alteration, exhibiting in some cases (Plate XIII, Fig. 4) a fibrillar arrangement. A most interesting feature of the dendrite is the nodules or gemmules which develop along its periphery. By the Golgi technique they stand out like pin-head prolongations or knobs. The presence of these gemmules on the dendrites has been denied by Hill (1896), while the other investigators regard them as very important and constant structures in certain forms of nerve-cells (Van Gehuchten, 1897, Cajal, and others). Still another interpretation has been made by Demoor (1896, 1898), who considers the moniliform appearance of the dendrite as a condition in which the gemmules are partially retracted and regards them as important for the normal activity of nerve-cells. I agree with the view which regards these structures as a constant character of certain forms of the nerve-cells. This knob-like structure can be seen not only in specimens prepared by the Golgi technique, but also in those prepared by my own method. In this case we can see clearly the internal structure of the gemmules and their relations to the main body of the dendrite. The gemmules are nothing more than a local extension of the ground substance of the dendrites, and a more or less modified reticulum can be seen within them in many cases (Plate XIII, Fig. 4). It is difficult, however, in some instances to distinguish the gemmules from the surrounding structures, when a large number of the neurosomic chains forming the axone terminals surround the dendrite very densely. Careful observation shows that the neurosomes in the gemmules stain less deeply than those forming the terminals. The accumulation of neurosomes to form gemmules is shown in Fig. 4, which has been drawn from the cells in the cerebral cortex of the adult white rat.

#### IV. TERMINATION OF THE AXONE ON THE DENDRITES AND CELL-BODIES

1. *Termination of the axone on the cell-body.*—The actual termination of the axone on the cell-body as well as a diffused network of the nerve-fiber terminals surrounding the cell-body and forming the so-called "pericellular network" has been



observed by Semi Meyer (1896), Held (1897), Ramon y Cajal (1899), Golgi and his students, and others.

In certain kinds of neurones the present writer has also been able very clearly to see these phenomena in his preparations. The cerebellar cortex is a most favorable locality in which to see the termination of the axones on the cell-body. It is a well-known fact that the Purkinji cells are surrounded by the terminals of the collaterals of the so-called basket cells, located in the molecular layer. Fig. 5 (Plate XIV) illustrates these terminations. In this figure the Purkinji cells are represented in sepia and the axone endings by a deep red. As can be seen, a large number of the neurosomes appear surrounding the basal portion of the cell-bodies together with their axones, and form a basket; while the upper part of the cell-body is in contact with a small number of the neurosomes along the cell-wall. According to the existing view, the basket-forming fibers are derived only from the collaterals of the axones of the cells which lie in the molecular layer. Contrary to this view, the writer believes that the fibers which form the basket have at least two sources of origin: that is, one from the molecular cells and the other from the so-called moss-fibers. This conclusion was drawn from the following evidence: by examining Fig. 5 (Plate XIV) one can easily see that the main part of the fibers which form the basket including the basal portion of the Purkinji cells descend toward the medullary layer and become continuous with some of the fibers in that layer. In other words, some of the fibers which enter into the granular layer enlarge very much and ascend as far up as the Purkinji cell layer, where they surround the latter very intimately and form the so-called "basket" in company with the descending collaterals from the cells in the molecular layer. In the same figure the sections of the main trunks, as well as the lateral branches of the moss-fibers in various planes, are shown distributed throughout the granular layer. In many cases these cross-sections of the moss-fibers are surrounded by the neurosomes which stain lightly. These structures, formed by the two kinds of the neurosomes, correspond probably to the glomeruli; and the neurosomes which stain lightly are identical with those which form the dendritic branches of the granular cells, while the rest of the neurosomes are those which form the moss-fiber.

An appearance similar to the basket of the Purkinji cells has been observed by the writer in the case of the cells in the Ammon's horn. Fig. 4 (Plate XIII), which has been drawn from the cells in the Ammon's horn, in the adult white rat shows the basal portion of the cell-body densely surrounded by the axones of another neurone forming a pericellular network.

The termination of the nerve-fibers on the cell-body in the corpus trapezoideum has been described by several investigators, especially by Held (1895). In the case of these neurones, according to him, the terminals of an axone come into contact relation with the cell-body of another neurone, yet one can always make out where the protoplasm of one neurone ends and where that of the second begins. Further, the line of demarkation is more refractive than the adjacent protoplasm. He finds, however, that

this refractive limiting line is not demonstrable in the adult and comes to the conclusion that during the processes of growth the protoplasm of the related neurones fuses.

As is stated by Held, the cells in this locality are very favorable for the study of the termination of the axones. As Fig. 6 (Plate XIV) shows, the terminals of an axone come in contact with the cell-body along a groove or an elongated depression. This groove on the cell-surface may coincide with the refractive area of Held. In most cases more than one axone terminates on a single cell-body. Fig. 6 was drawn from the material taken from a young white rat having a body-weight of 4.5 grams. In this stage a number of axones terminate on each cell-body. No special area for the termination of the axones appears, since they are found in all regions of the cell-body, sometimes at the center and sometimes at the end of it. In all cases the terminals of the branches present mere contiguity to the cell-surface, and neither fusion of one with the other nor a pericellular network of the axones is found. It is to be noted that these observations apply to the white rat, while the observations of Held were made on the rabbit. Whether the cell-body in the rat becomes fused with the axones in adult life has still to be determined.

A relation between axone and cell-body similar to that in the corpus trapezoideum can be observed in the ventral horn cells of the spinal cord. Fig. 2 (Plate XIII), which was drawn from the preparation of an adult white rat, illustrates this. In this figure the cell-body is represented by sepia while the axone endings are colored an intense red.

2. *Termination of the axone on the dendrites.*—As has already been mentioned, the gemmules are lateral extensions of the dendrites, and their essential structure is the same as that of the dendrites. A careful observation of the preparation shows that the axones in most cases surround the dendritic branches and approach so closely to the gemmules that these two structures often come into contact. As Fig. 4 (Plate XIII) shows, the cell-bodies in the Ammon's horn are densely surrounded by the axones and some of the latter climb along the surface of the dendrites and there come into contact with the gemmules. This relation is even more clearly shown in the cerebellar cortex. It is already known that the dendrites of the Purkinji cells are densely surrounded by several kinds of the axones; namely, those of the granular cells, those forming the climbing fibers, and those which form the moss-fibers. The axone terminals which surround the dendrites come, in most cases, actually in contact with the latter. Fig. 5 (Plate XIV) shows such a relation between the two processes where the dendrites are represented in sepia, while the axones are colored an intense red.

The so-called "glomeruli" formed by the axones and dendrites form the most favorable structure for the study of an intimate relation between the two processes. This structure is found best developed in the olfactory-bulb and less developed in the granular layer of the cerebellar cortex. The olfactory glomeruli in Fig. 3 (Plate XIII) were

drawn from that of the new-born white rat having a body-weight of 4.5 grams. For convenience the axones are represented in red, while the dendrites are in yellow. Although the olfactory glomeruli are of very complicated structure, owing to an intricate arrangement of the two kinds of the processes, yet, after knowing the character of the axone and dendrite as determined by the neurosomes in them, one can easily see that in many cases a single long and apparently continuous filament is composed of two differently characterized parts; that is, the neurosomes in one portion are much larger and stain more deeply than those found in the other portion. In other words, these apparently continuous lines are composed of two different structures, the axones and the dendrites. In the case of the glomeruli in the granular layer of the cerebellar cortex, continuous filaments formed from two sorts of processes, as observed in the olfactory glomeruli, were not found, but a mere contiguity of the two processes, such as is noticed in the dendrites of the Purkinji cells and the axones which surround them, was all that could be observed.

#### GENERAL REMARKS

The history of the investigations on the neurone has been beautifully summarized by Goldscheider and Flatau (1898), Barbacci (1899), Barker (1899), Robertson (1899), Soury (1899), and Van Gehuchten (1900), but in order to show the bearing of our own observations, it will be necessary briefly to review the more important theories concerning the neurone.

According to the most prevalent view, the "neurone" or the nerve-cell with all its processes may be regarded as an independent element, from the anatomical standpoint; consequently the entire nervous system is an aggregation of those independent elements. This view was first brought out by Waldeyer (1891). He says:

Das Nervensystem besteht aus zahlreichen untereinander anatomisch wie genetisch nicht zusammenhängenden Nerveneinheiten (Neuronen). Jede Nerveneinheit setzt sich zusammen aus drei Stücken: der Nervenzelle, der Nervenfasern und dem Faserbäumchen (Endbäumchen). Der physiologische Leitungsvorgang kann sowohl in der Richtung von der Zelle zum Faserbäumchen als auch umgekehrt verlaufen. Die motorischen Leitungen verlaufen nur in der Richtung von der Zelle zum Faserbäumchen, die sensiblen bald in der einen, bald in der anderen Richtung.

This view of Waldeyer, or the neurone doctrine, has been somewhat modified since Held, in 1896, noticed in some neurones an actual contiguity of the axones both with the cell-bodies and dendrites. Held's observation was very soon confirmed by a number of investigators and was further extended to another group of neurones. Held's observation, however, does not oppose the neurone doctrine, for he notices a mere contiguity of the axones with the cell-body and dendrites and not an organic continuation of one into the other. In the following year Bethe (1897) published an article in which he claims that the nerve-cells and dendrites contain a great number of primitive neuro-fibrils which run toward the axone and form the nerve-fiber. That is, the nerve-fiber is composed of these primitive fibrils. He believes, further, that the fibrils

of one neurone enter into the nerve processes of other neurones, and thus two neurones become continuous by means of these primitive fibrils. The observations were made on crustacea. Apathy's (1897) observation on the lower animals (annelids) contradicts radically the neurone doctrine, for he was able to follow the primitive neuro-fibrils which come from one ganglion cell and enter into the cell-body of another element, where they become fused with the protoplasm. Anastomosis of the axones with dendrites has been observed by several other investigators, for instance, Ballowitz (1893), Heymans and Demoor (1894), and others; but in these cases always in the peripheral system. Thus Gerlach and Golgi's hypothesis of a diffuse network of the nerve-processes has been revived through a more careful investigation of modern neurologists.

It is impossible at the present moment to say which of these views is correct, since we do not know absolutely which technique shows the tissue in most nearly normal condition. But after examining the results obtained by several investigators, it seems to be quite reasonable to say that Golgi's silver-nitrate technique is not effective enough to bring out the minute structures of the neurones, and, further, it acts on the tissue so irregularly that in some cases even the same tissue in the same condition presents a widely different appearance. In addition, the ordinary silver-bichromate method does not show the internal structure of the neurones. Consequently, for the purpose of this discussion, results obtained by Golgi's technique can hardly be considered as at all conclusive.

As has already been mentioned, the nerve-cells in the white rat present a fibrillar structure owing to the parallel arrangement of the neurosomes. This structure, however, is merely a modified reticulum which has been very much elongated. In some cases several of these parallel lines of the elongated meshes combine together and form very thick strands. Further, these united filaments or strands are found throughout the cell-body, forming a very complicated network. In the case of the dendrites these united filaments are noticed most frequently. Now, comparing these figures with that of Golgi's endocellular network previously mentioned, one might expect the two figures to be identical, for this anastomosis of combined filaments in the cells in the white rat occurs only around the nucleus and in the neighborhood of the axone hillock, not in the hillock itself, and never occurs along the periphery of the cell-body, where wide meshes of a polygonal shape are alone visible. Golgi's endocellular network has a similar distribution within the cell-body. A similar arrangement has been observed in the cells of cerebral and cerebellar cortex. The only difference between Golgi's results and those of the present writer is that Golgi's network is much simpler than the latter and does not show any minute meshes formed by delicate filaments. This difference is due very probably to an insensitiveness of Golgi's technique, so that it does not bring out these minute structures.

It has been suggested by some investigators that Golgi's endocellular network might represent the system of the intracellular canaliculi of Holmgren. If Golgi's

endocellular network is really homologous to the canal system described by Holmgren, it should occur along the cell-periphery where this canal system is most abundant as well as larger in diameter. Further, as has been pointed out by Soukanoff (1902), these two structures do not show any similarity in appearance.

The present writer thinks also that the anastomosis of fibrils of Apàthy within the cell-body may be a homologous structure to both Golgi's endocellular network as well as to the network here described. Judging from its manner of distribution and position in the cell-body, they are nearly identical with one another. Slight variation in the structure depends upon the tissues taken from animals which are widely different. The more complex anastomosis of Apàthy are due to the greater accuracy of technique.

It seems to me, therefore, that the fundamental structure of the ground substance in the nerve-cell shows reticular arrangement, which, however, becomes sooner or later elongated, and thus the fibrillar appearance in the axone hillock, axone, and dendrite, and the complicated anastomosis in the cell-body, are brought out in the way previously described.

As will be seen from the previous description, certain nerve cell-bodies, such as Purkinji's, the pyramidal cells and the cells in the corpus trapezoideum and ventral horn of the spinal cord, are densely surrounded by the terminals of the axones, and in some cases not only surrounded, but some of the axones terminate on the cell-body and become contiguous with it. Further, the dendritic processes, especially the gemmules, are as a rule very densely surrounded by the axone terminals. In all these cases, however, those two kinds of structures are merely in contact with each other, and the present writer was not able to see any actual continuity between the two. Even in the case of the olfactory glomeruli, where the axones and dendrites unite and form a single filament, these two structures can nevertheless be clearly distinguished. I conclude, therefore, that, although the two structures appear continuous with one another, nevertheless the junction point can always be recognized by the differences in structures in either side of it.

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## EXPLANATION OF THE PLATES

## PLATE XIII

FIG. 1.—Spinal ganglion cell from a cervical ganglion of the adult white rat. Reddish-brown (surrounding the cell-body) represents the capsule which is composed of connective tissue. Several sheath-nuclei as well as a cross-section of a capillary containing the blood corpuscles are shown. Within the cell the larger red granules represent the neurosomes, while the smaller granules of the same color represent the cyto-microsomes. The nucleus which is represented also in red contains a single nucleolus (blue) and a large number of the oxyphile granules (red). Nissl bodies are represented in blue. The location of the axone hillock is indicated by the absence of the Nissl bodies.

FIG. 2.—A motor cell from the ventral horn of the spinal cord of the adult white rat. Lighter red represents the body of the motor cell which contains a spherical nucleus (slightly darker red) at the center. The dots of an intense red represent the neurosomes which form the axone endings. They terminate on the surface of the cell-body.

FIG. 3.—An olfactory glomerulus of the new-born white rat, having body-weight of 4.5 grams. Red lines represent the olfactory nerve-fibers, while the yellow lines represent the dendritic branches of the mitral cells. The neuroglia nuclei are represented in blue.

FIG. 4.—Cells from Cornu ammonis of the adult white rat. The larger cell (on the left side) shows a mode of termination of axones which are composed of a large number of the neurosomes. The small cell represents the internal structure of the cell-body. The neurosomes are represented by red dots, while the nucleus in which is a single nucleolus (blue) and a large number of the oxyphile granules (red) is outlined in red. Blue in the cytoplasm represents the Nissl bodies.

## PLATE XIV

FIG. 5.—The cerebellar cortex of the adult white rat. Purkinji cells and their dendrites are represented in sepia. Nerve-fibers and neurosomes are represented in red. Nuclei in both granular and molecular layers as well as the blood capillaries are represented with black.

FIG. 6.—Cells from corpus trapezoideum of the young white rat having body-weight of 4.5 grams. Each nucleus contains a single nucleolus (blue) and a large number of the oxyphile granules (red). Blue in the cytoplasm represents the Nissl bodies. Red lines (heavier) represent the terminals of the axones while the lighter lines in the outside of the cell-bodies represent either the neuroglia fibers or fine nerve-fibers.

All the figures were drawn from restrained preparations (see the technique in the text) by free hand, using Obj.  $\times$  OC, 4, Zeiss, except Fig. 5, which has been drawn by using Obj. 4, 0 mm.  $\times$  OC. 4, Zeiss.

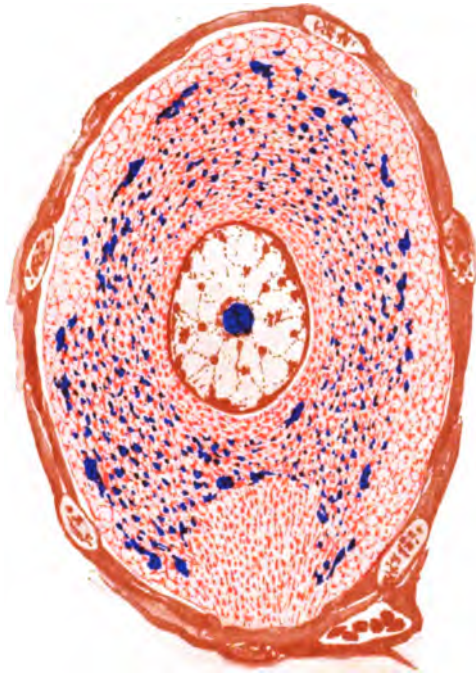


FIG. 1



FIG. 2

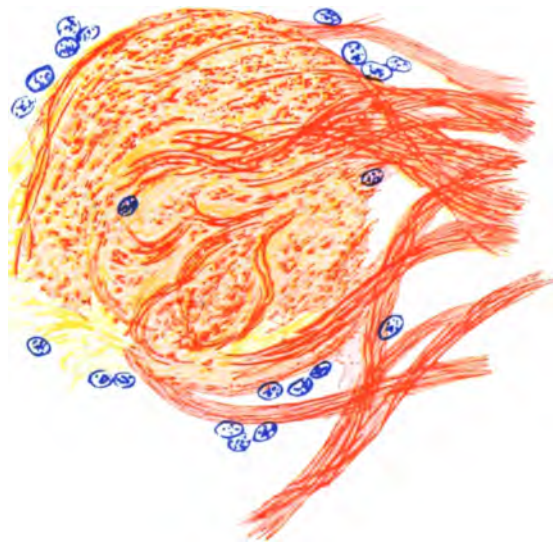


FIG. 3

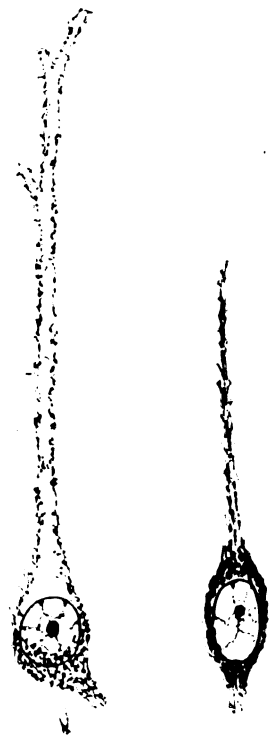


FIG. 4

- FIG. 1. SPINAL GANGLION CELL FROM WHITE RAT  
 FIG. 2. VENTRAL HORN CELL FROM WHITE RAT. AXONE TERMINATIONS  
 FIG. 3. OLFACTORY GLOMERULUS. NEWBORN WHITE RAT  
 FIG. 4. CELLS FROM CORNU AMMONIS OF WHITE RAT. GEMMULES





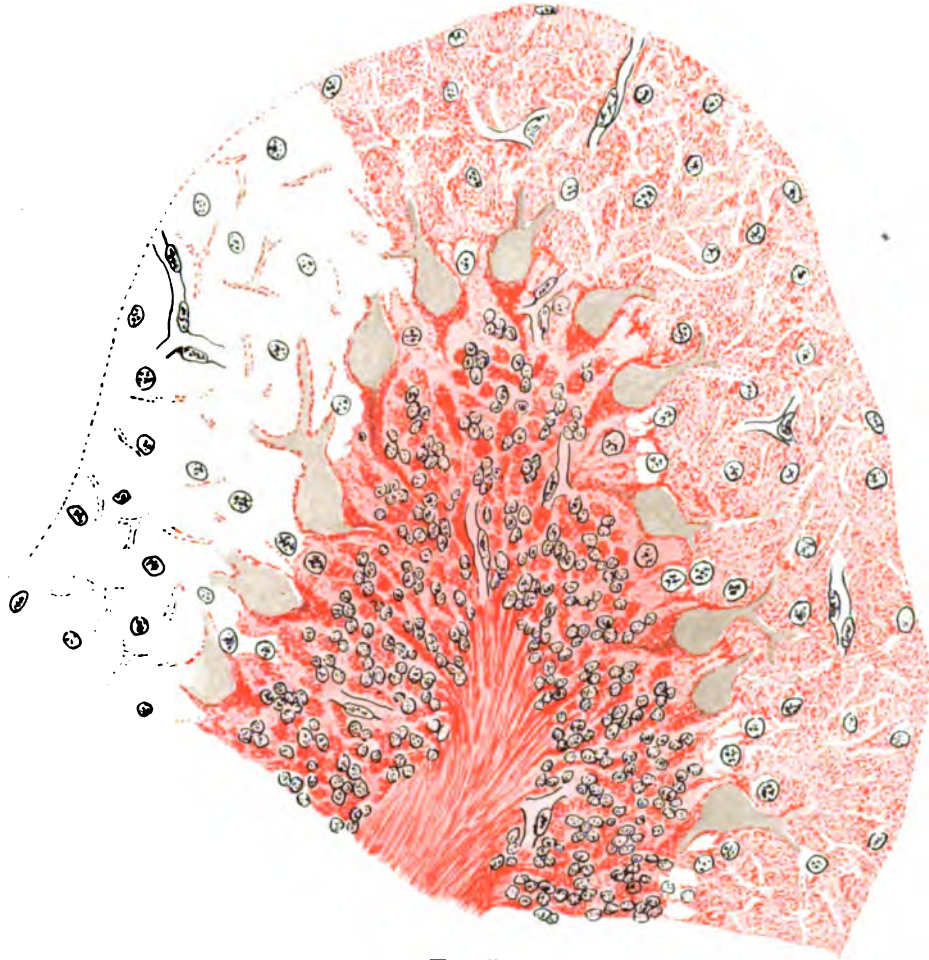


FIG. 5

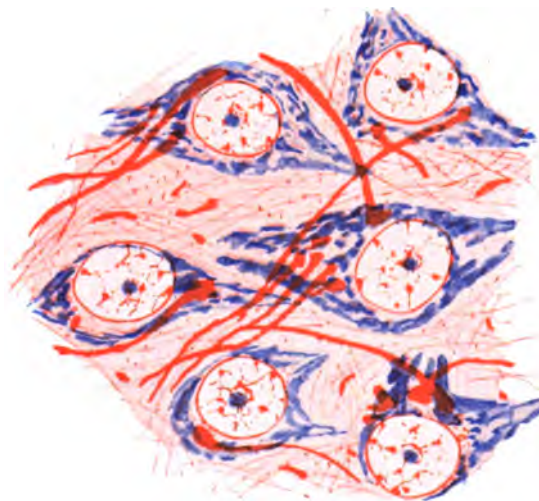


FIG. 6

FIG. 5. CEREBELLAR CORTEX OF WHITE RAT. AXONE TERMINATIONS.  
FIG. 6. CELLS FROM CORPUS TRAPEZOIDEUM OF WHITE RAT. AXONE TERMINATIONS.



# **THE PHYLOGENY OF ANGIOSPERMS**



# THE PHYLOGENY OF ANGIOSPERMS

JOHN M. COULTER

THE views presented in this paper are in the main based upon numerous investigations conducted by members of the botanical staff and graduate students. The accounts of these investigations, extending through a period of six years, have been published from time to time, chiefly in the *Botanical Gazette*, but their bearing upon the problem of the phylogeny of Angiosperms has never been presented. It would be confusing to cite the literature involved in this presentation, as it would mean an extensive bibliography and is in the main familiar to students of the Angiosperms. The purpose is to present in as compact form as possible the bearings of our present knowledge upon a problem of great obscurity. The phylogeny of any great group will probably always remain a baffling problem, incapable of actual demonstration, and yet theories of phylogeny serve to co-ordinate knowledge and stimulate investigation. It should be stated that when similarity of structure was taken to be a sure indication of genetic relationships, the problem promised an approximate solution. But since it has been proved that similar structures may develop independently, the difficulty of solution has become apparently insurmountable.

The first phase of the problem has to do with the common or independent origin of the Monocotyledons and Dicotyledons. The current view assumes their monophyletic origin, a view based largely upon the great uniformity of the peculiar development of the female gametophyte. It is argued that the independent origin of such exact details of development and structures is inconceivable. The peculiar female gametophyte of Angiosperms, however, has been found to vary enough to indicate that it is an extreme expression of tendencies evident in the heterosporous Pteridophytes and Gymnosperms; in other words, an ultimate result of heterospory. Further, nothing is more clear than that heterospory has originated independently in several plant groups; and the assumption that its ultimate expressions, the seed and the angiospermous female gametophyte, have been reached by only one line seems more than improbable.

This somewhat theoretical objection to the current argument in favor of the monophyletic origin of Angiosperms is strengthened by certain fundamental differences between the Monocotyledons and Dicotyledons. The differences in the development of the embryos of the two groups are hard to reconcile upon the theory of monophyletic origin. Recent investigations of all of those Dicotyledons that have been called "pseudo-monocotyledonous," on account of their apparently terminal cotyledon and lateral stem-tip, have shown a normal dicotyledonous embryology with more or less complete abortion of one of the cotyledons and displacement of the stem-tip through the development of the functional cotyledon. Again, the differences in the structure

of the stem and in the character of its vascular bundles are far more difficult to connect genetically than to refer to a polyphyletic origin, with all that that implies. One of the strongest arguments against the monophyletic theory comes from historical testimony. The "Proangiosperms" of the Lower Cretaceous, so far as known, appeared associated with undoubted Monocotyledons, and merged gradually into recognizable Dicotyledons, without indicating any relationship to the Monocotyledons. The emerging of Dicotyledons from this vague group either indicates that they originated independently, or that the Proangiosperms were transition forms between Monocotyledons and Dicotyledons. The latter alternative is inconceivable, especially since the most primitive Dicotyledons are now recognized to be more primitive than any of the Monocotyledons.

It is of interest to note that recent anatomical investigations contradict the current view that Monocotyledons are the more primitive, and Dicotyledons derived from them, and show, so far as the development of the vascular system is concerned, that Monocotyledons are derived from Dicotyledons in case they have a common phylogeny.

All the testimony available, morphological, historical, and anatomical, seems most consistent when interpreted in favor of the polyphyletic origin of Angiosperms.

The second phase of the problem is to determine whether the Angiosperms have been derived from Gymnosperms or from Pteridophytes. The general question is the same whether one believes in their monophyletic origin or not. The older view is that Angiosperms are phylogenetically related to Gymnosperms, and *Gnetum* has been regarded as the nearest living representative of a transition condition between Gymnosperms and Angiosperms. The argument is based upon such angiospermous characters in *Gnetum* as the absence of archegonia, the organization of eggs while the gametophyte consists only of free cells, the presence of a perianth and true vessels, and the dicotyledonous leaves. This showing is certainly strong, and especially in the structure of the embryo sac does *Gnetum* show characters that may well illustrate a stage in the Angiosperm phylum; but that it actually represents the group from which the Angiosperms were derived seems unlikely. In fact, the historical argument against such a claim is very strong, for there is no evidence that *Gnetum* or allied forms existed among the numerous Angiosperms of the Cretaceous and Tertiary. If it were related in any way to the origin of so dominant a group as the Angiosperms, it seems probable that it would have left some evidence of its existence. Nor is it of special avail to claim that fossil Gnetales may be found in the tropics or in the Southern Hemisphere, for the great uniformity of climate during early periods has left the records of tropical vegetation in the temperate and even boreal regions of today; so that the strata of the tropics are not likely to reveal prominent types of vegetation unrepresented in the strata of temperate regions.

The argument from the presence of a "perianth" is particularly vulnerable, since the structure so-called merely represents the bracts common among Gymnosperms, and

the most primitive Angiosperms have no perianth. Further, the presence of true vessels in the secondary wood is an argument as much in favor of the origin of Angiosperms from certain heterosporous Pteridophytes as from *Gnetum*. Every indication points to the conclusion that *Gnetum* is a highly specialized and comparatively recent member of the Gymnosperm phylum, and as such could not have given rise to the Angiosperms.

If the Gymnosperms are not the ancestral forms of the Angiosperms, the direct derivation of the latter from Pteridophytes becomes a matter of course. Perhaps it was natural to turn at first to the heterosporous Pteridophytes, and among them the only forms that could seem to be within the range of probability are *Selaginella* and *Isoetes*. The latter has been persistently associated with the origin of the Monocotyledons, especially in connection with the former idea that Monocotyledons are the primitive Angiosperm stock. Any supporter of this view now would be almost forced to maintain the polyphyletic origin of Angiosperms. The most striking resemblance of *Isoetes* to the Monocotyledons occurs in the embryo, in which the single cotyledon is terminal and the stem-tip lateral. A thorough investigation of *Isoetes*, however, has developed so many difficulties in the way of accepting it as related in any way to the Monocotyledons that the theory must be regarded as untenable.

The only possible alternative as to the origin of Monocotyledons, in case they have arisen independently of the Dicotyledons, is to regard them as the end of a heterosporous line that developed independently from the eusporangiate Filicales, whose Pteridophyte members are extinct. Since several independent heterosporous lines are already recognized, it is not at all necessary to connect any seed-plants with living heterosporous Pteridophytes.

More important, however, seems to be the determination of the origin of the Dicotyledons, whether they represent an independent phylum or the primitive angiospermous stock. The fact that they emerged from the so-called "Proangiosperms," which were largely displayed in the earlier part of the lower Cretaceous, seems to be fairly well established. The question, therefore, has to do with the origin of the Proangiosperms. They do not seem to warrant the belief that they represent a common stock from which both Monocotyledons and Dicotyledons have been derived, for the Monocotyledons are believed to have existed in unmistakable forms before the large assemblage of Proangiosperms gave rise to unmistakable Dicotyledons. Still less conceivable is it that Proangiosperms represent the transition forms from Monocotyledons to Dicotyledons, for nothing in their known structure seems to suggest such a view. That they were derived from *Gnetum*-like forms is discredited by the fact that there is no sure record of the existence of *Gnetum* at such an early period, and to have given rise to such an assemblage of forms it must have been a conspicuous group.

If we turn to the earlier groups that were sufficiently prominent and at all suggestive of having given rise to the Proangiosperms, we encounter the Coniferales, Cycadales, Lycopodiales, and Filicales. The Gymnosperm origin of Dicotyledons



seems to be most unlikely with the exclusion of *Gnetum*. At the same time, it might be claimed that Dicotyledons represent an independent line from the Gymnosperm stock, that advanced in the same direction and much farther than did the *Gnetum* line; but all the essential morphology of the Gymnosperms is less favorable to such an origin than is that of the heterosporous Pteridophytes.

The Lycopodiales certainly deserve serious consideration in this connection. The structures of *Selaginella* are about as suggestive of Dicotyledons as those of *Isoetes* are suggestive of Monocotyledons, the embryo being as distinctly dicotyledonous as that of *Isoetes* is monocotyledonous, and our study of *Selaginella* has shown the strikingly seed-like character of the megasporangium. But a seed may be attained by any heterosporous line, and *Selaginella* and even its ancient *Lycopodium* stock have too many peculiarities to be considered seriously as ancestral types of Angiosperms.

The only other alternative is that mentioned in connection with the origin of the Monocotyledons, namely the derivation of the Proangiosperms as an independent heterosporous line from the abundant ancient eusporangiate Filicales, and this view is supported by anatomical testimony.

It is becoming increasingly evident that the great marattiaceous plexus of the Palæozoic probably gave rise to several heterosporous lines, one or more of which have certainly been responsible for the Gymnosperms, and others have led to the Angiosperms. As heterospory may lead to seed-formation in any line, it is inconceivable that only one or at most two of the numerous heterosporous lines have attained seed-production. It is more probable that the Angiosperms have arisen from the Marattia-like ferns in several independent lines; that the group known as Angiosperms is determined by its attainment of seed-production rather than its monophyletic origin; but that in a certain sense it has a common phylogeny and hence numerous characters in common.

A summary of these conclusions may be stated as follows:

1. The Monocotyledons and Dicotyledons comprise at least two independent angiospermous lines, and do not represent a single phylum.
2. No Angiosperm phylum has been derived from the Gymnosperms or from living heterosporous Pteridophytes.
3. All Angiosperm phyla have been derived as independent heterosporous lines from the ancient eusporangiate Filicales, which also gave rise to the Gymnosperms.
4. Several Angiosperm phyla probably arose independently from the marattiaceous plexus of the Palæozoic.
5. If Angiosperms have a monophyletic origin, which seems very unlikely, it seems clear that the Monocotyledons have been derived from the more primitive Dicotyledons.

## **STUDIES IN FAT NECROSIS**



## STUDIES IN FAT NECROSIS

H. GIDEON WELLS

LANGERHANS<sup>1</sup> made the first actual attempt to learn the cause of the changes of fat necrosis in 1890. Before that time Balser (1882),<sup>2</sup> who first called attention to the condition, and Chiari (1883)<sup>3</sup> had attempted to reach conclusions by study of the histology of the lesions. The former decided that overgrowth of fat tissue was at the bottom of the trouble, thinking that the new fat cells which he described as growing about the periphery shut off the blood supply of the center of the focus. Chiari took an exactly opposite view. Observing fat necrosis in the bodies of marantic patients, he considered that lack of nutrition during cachexia was the cause. From his histologic studies he concluded that the process was not at all different from retrogressive changes seen in other organs and there designated as simple necrosis and fatty degeneration.

Langerhans's first studies were of a histologic and micro-chemic nature. He observed that the necrotic masses did not float like masses of stearin, but sank quickly to the bottom. On teasing out they were seen to consist of characteristic needles of fatty acids, a few small oil droplets, and many flakes of varying size, the last being responsible for the high specific gravity. Further evidence of the change from the normal fat that had previously occupied the affected area was furnished by staining with osmic acid, which merely yielded a dirty, grayish-brown coloration. Addition of concentrated sulphuric acid to sections from which the fat had been removed with alcohol and ether caused complete solution of the heavy flakes. If this treatment was followed by the addition of water, and the specimen allowed to stand until the following day, numerous characteristic crystals of calcium sulphate settled out. From these observations he concluded that, since both calcium and fatty acids existed in the necrotic areas, they were probably combined. Further evidence was furnished by the insolubility of the calcium salts in hydrochloric acid, which showed that they were not carbonates or phosphates, and the insolubility of the fatty acids in boiling ether, which showed that they were not free. At the time Langerhans wrote the knowledge of the history of fat in metabolism was less advanced than now, and he sought support for his assumption of the formation of calcium soaps in the observations on this process as it occurs in adipocere. With this exception the chief illustration of such a process was the description of retrogressive metamorphosis in lipomas by Virchow,<sup>4</sup> who said that in these tumors the fat is occasionally saponified, combines with calcium and magnesium, and together with earthy phosphates forms a crumbling, mortar-like mass. By

<sup>1</sup> *Virchow's Archiv*, Vol. CXXII (1890), p. 252.

<sup>2</sup> *Ibid.*, Vol. XC (1882), p. 520.

<sup>3</sup> *Prager medicinische Wochenschrift* (1883), No. 30, p. 285; No. 31, p. 299.

<sup>4</sup> *Krankhafte Geschwülste*, Vol. I, p. 393.

studying fresh specimens, Langerhans sometimes found foci in the middle of a fat lobule consisting of fat cells full of fatty acids without calcium salts, showing that the splitting occurred first and the union with calcium later. His conception of the process after these studies he states as follows:

To collect these observations briefly, it develops that the multiple necroses of fat tissue begin with decomposition of the neutral fat contained within the cells; the fluid constituents are eliminated and the solid fatty acids remain behind. The latter combine with calcium to form calcium fatty-acid salts. The entire lobule or several neighboring lobules form a dead mass, which has the destroyed separated from the living tissue through a demarcating inflammation.

The part played by the fat cell itself in the necrosis was puzzling. He found that the nucleus did not lose its affinity for stains until after decomposition of the contained fats. He says:

Although there is no demonstration of a primary injury to the cell, I cannot hazard the conclusion that the physiological function of the cell is intact at the beginning of the decomposition of the fat, because we know so little of the rôle of the cells in the building and taking up of fat. It would perhaps be simplest to assume that in metabolism somewhere within or about the cell a harmful substance appeared and accomplished the decomposition of the fat droplets, but, unfortunately, every observation fails to support this.

The frequent association of fat necrosis with lesions of the pancreas, the frequent localization of the process to the fat about the pancreas, and the similarity of the fat-splitting that he had described with that of pancreatic digestion, led Langerhans<sup>5</sup> to study the relation of this organ to the disease experimentally, and in 1891 he described his methods and results. He made an extract of the pancreas of freshly killed rabbits by rubbing the gland in a mortar with splintered glass, under aseptic precautions. This was injected after filtration into twelve animals—nine rabbits and three dogs—and in one rabbit a single spot of typical fat necrosis appeared in the perirenal fat. From this one success he believed that the pancreatic juice was the cause of the fat necrosis, but of course he could not obtain any clear conception of the process.

Since that time a large variety of experiments have been tried in the study of fat necrosis, most of which have simply added a new method by which pancreatic juice can be made to enter the peripancreatic fat, or the subcutaneous fat (Williams) and the abdominal cavity, when, all are agreed, fat necrosis frequently, but not invariably, results. Experiments in which extracts or dried preparations of the pancreas have been used, rather than the secretions of the living gland, have been made by Jung and by Dettmer.

Jung<sup>6</sup> placed a gelatin capsule containing "trypsin" in the abdominal cavity of one rabbit, and pieces of dog's pancreas in three others. All showed fat necrosis, which he thought was due to the fat-emulsifying and fat-splitting ferment, but the greater part to the proteid-splitting enzyme. The experiment upon which Jung based his idea of the importance of trypsin was the one mentioned first, in which a prepara-

<sup>5</sup> *Festschrift zur Feier des 71. Geburtstages Virchow's.*

<sup>6</sup> *Dissertation, Göttingen, 1895.*

tion of pancreas in a gelatin capsule was placed in contact with the omentum. As this preparation, which he calls "trypsin," seems to have been merely dried and pulverized pancreatic tissue, his assumption of a pure tryptic action is quite unwarranted, since the other ferments are not excluded.

Dettmer<sup>1</sup> first tried the effect of causing pancreatic juice to enter the abdominal cavity, which was accomplished variously by ligating the pancreatic duct, or the vessels, or both, by placing pancreas tissue in the abdominal cavity, and by injecting an extract of fresh pancreas. As these caused fat necrosis, he endeavored to find what ferment was responsible, to which end he injected trypsin into still other animals. As trypsin alone did not cause fat necrosis, he concluded that the fat-splitting ferment must be responsible. The trypsin used by Dettmer was quite a different article from that used by Jung, and he describes it as follows:

The trypsin used by us (from the firm of Merck in Darmstadt) was studied thoroughly after it was softened in water; it showed no trace of organic structure, but had a more crystalline appearance; it was designated by the firm as the proteid-splitting substance, in contrast to another preparation, called pancreatin, which contains all active elements of the pancreas. A test of our preparation for a saccharificant action gave a negative result, likewise the test for fat-emulsionizing property. Our preparation was therefore pure trypsin. We obtained with our pure preparation only hemorrhage, no fat necrosis in the Balser-Langerhans sense.

It will be observed that no test for lipolytic activity was made. The experiments performed by Dettmer were as follows:

(Experiment 1.) Injected into the peritoneal cavity of a cat "a large quantity of a watery solution of trypsin." Thirteen days later, the cat having remained well, the abdomen was opened aseptically and nothing abnormal found. About one gram of powdered trypsin was then sprinkled upon the omentum and intestines of this cat (Experiment 3). The animal died in less than twenty-four hours, and autopsy showed great hyperæmia and many hemorrhages in the omentum, but the fat itself "showed no traces of alteration." There was no evidence of peritonitis. Experiment 2 was a duplicate of Experiment 1, with likewise a totally negative result. This animal then had about 0.5 grams of trypsin placed in the abdomen, after which the wound was closed (Experiment 4). Death occurred seven days later. Autopsy showed no peritonitis; the fat was entirely unaltered, but there were a few small hemorrhages in the omentum. The parts of the intestine that had come in contact with the trypsin were superficially eroded. No histologic examination is mentioned. Dettmer's conclusions are as follows:

These experiments show beyond question that the proteid-splitting ferment, the trypsin, is not able to produce fat necrosis. But, as fresh pancreatic juice is always able to cause fat necrosis in living fat tissue, there remains nothing more possible except that this action is due to the third ferment contained in pancreatic juice, the fat ferment.

Except for the series of experiments just mentioned, the numerous experiments performed by many different investigators have, for the most part, added nothing to the important matter of how the pancreatic juice causes this peculiar form of necrosis,

<sup>1</sup> Dissertation, Göttingen, 1895.

and what constituent of the juice is the active one. Their results have shown that fat necrosis can be obtained in the following ways: By injecting aqueous extracts of the fresh gland; by transplanting pieces of pancreas; by ligating the pancreatic duct, with or without laceration of the organ to facilitate escape of the pancreatic juice; by ligating the veins leading from the pancreas, with or without injury to the gland or ligation of its duct; by injection of necrotizing or infectious substances into the gland itself or into its duct; by cutting the duct or bisecting the organ, so that its secretion escapes into the surrounding tissues; by binding between glass rods, and other forms of trauma. All of these methods have close relation to the conditions of trauma, hemorrhage, and infection which ordinarily are the cause of fat necrosis as seen in man. These experiments, as well as clinical observations, taken altogether leave no doubt that pancreatic juice, and it alone, is the cause of fat necrosis, bacteria having been excluded by many negative cultural experiments. Further than this they tell little.

In contrast to them is the work of Flexner,<sup>8</sup> in which it was attempted to prove definitely the relation of pancreatic lipase to fat necrosis, which relation had, since Langerhans's one successful experiment, generally been assumed to be a positive one. Flexner's studies were directed to prove the presence of lipase in the areas of necrosis in both human and experimental cases, and its absence in the normal fat tissue. His method is described as follows: A neutral fat was prepared from fresh butter by dissolving it in ether, adding a few drops of NaOH solution, and washing out with water. The separated ethereal solution of neutral butter was evaporated over a water bath, and the tests were made at once. For this purpose were employed (1) a piece of the pancreas itself, (2) one or more focal necroses, and (3) a piece of adipose tissue equal in size to the pieces of pancreas or necroses, this piece being taken from a distance from the necroses. The pieces of tissue were placed in 90 per cent. pure alcohol for from one-half to two hours, pressed in filter paper, re-immersed in the alcohol for a short period, again pressed and allowed to become air-dry. They were then cut into morsels, which were incorporated with the neutral fat in watch-glasses, which were then covered and placed in the thermostat at body temperature. The fatty acids liberated were demonstrated by the odor and by tincture of litmus, the reaction being sometimes obtained, it is stated, within six minutes. Fat necrosis was produced in cats and dogs by various methods leading to escape of juice from the gland, most satisfactory of which was combined ligation of the veins and laceration of the gland. In one case of fat necrosis Flexner was able to obtain a positive reaction in ten minutes from the necrotic tissue and from the pancreas, but not from the normal fat tissue. In animals killed on the second, third, and fourth days all gave evidence of fat-splitting enzyme in the necroses, but not in the normal fat. To test the time at which the lipase (steapsin) disappeared, pieces of necrotic tissue were excised aseptically from cats on the third day after the operation on the pancreas, and

<sup>8</sup> *Journal of Experimental Medicine*, Vol. II (1897), p. 413.

the animals allowed to live until the sixth and eighth days. The conclusions drawn from the experiment were:

(1) In peritoneal fat necrosis the fat-splitting ferment is demonstrable at certain stages of the pathological process; (2) it is present in greatest amount in the early stages and may disappear in the later ones when healing is well advanced; (3) although it cannot be affirmed that steapsin is the direct cause of the necrosis of tissue, such an assumption is rendered highly probable by its constant occurrence in the diseased areas, its absence from the healthy fat, and the nature of the pathological changes; (4) the escape of the pancreatic secretion into the peri- and para-pancreatic tissues is the origin of the necroses, and this escape is facilitated chiefly by lesions of the pancreas itself, but also by disturbances in its circulation.

Flexner also adds:

That necrosis of fat cells may result from other causes is certainly not excluded by these findings. The genesis of similar lesions found in the marrow of the bones by Ponfick, in the subcutaneous fat (Chiari) and pericardial fat (Balser, Chiari) is not immediately apparent. That micro-organisms may, in these cases, play a part is, in my opinion, very probable indeed.<sup>9</sup>

This valuable work of Flexner's, filling out the results of Langerhans and Dettmer, seemed to leave little doubt that fat necrosis is simply a matter of escape of pancreatic juice into the fat tissue about the gland and into the peritoneum, and the lipase (steapsin) acting upon the fat causes its splitting, which leads to the peculiar and characteristic foci. To be sure, Flexner appreciated the incompleteness of the demonstration and the many unsolved questions, but this has been generally overlooked, and at the present time the teaching is usually as above stated.

The evidence upon which is based the belief in the primary importance of lipase in the pathogenesis of fat necrosis, human and experimental, is as follows: (1) the microscopical evidence of fat-splitting, consisting in the demonstration of masses of crystals of fatty acids in the areas of necrosis, together with the disappearance of the fat from these areas; (2) the association of fat necrosis with the occurrence of free pancreatic juice, the known presence of lipase in this fluid, and the inability to produce fat necrosis with the tryptic ferment (Dettmer; the experiments of Jung seem to be of little or no value); (3) the demonstration by Flexner and by Opie of a fat-splitting ferment in the areas of necrosis, while it could not be demonstrated in the normal fat tissue.

The course of events is believed to be about as follows: (1) an injury to the pancreas by infection, trauma, occlusion of ducts (Opie), or what not; (2) escape of more or less normal pancreatic juice either free into the tissue or into the lymph stream; (3) dissemination of the fluid, in a manner not always easy to explain; (4) action of the lipase upon the fat cells, leading to splitting of their contained fat into glycerin and fatty acid; (5) glycerin diffuses, leaving behind the solid fatty acids; (6) necrosis of the cells; (7) eventually the fatty acids combine with

<sup>9</sup>A full corroboration is furnished by E. L. OPIE, *Contributions of the Pupils of William H. Welch* (Baltimore, 1900), p. 859, who, using Flexner's method, obtained reac-

tions with foci remote from the pancreas, namely the pericardial and subcutaneous fat, in animals in which widespread dissemination of the lesions had been produced.



calcium salts and are precipitated, while a new growth of connective tissue encapsulates the area and diminishes its size.

All this is simple and clear-cut, but unfortunately far from proved. This is particularly so when the part supposedly played by lipase is considered in the light of recent developments in the study of fat and fat metabolism. J. H. Kastle and A. S. Loevenhart<sup>10</sup> have demonstrated, not only that lipase is present in all the tissues of the body in which there is any utilization or storage of fat, but also that lipase acts reversibly upon fat and its components, building up fat from the fatty acid and alcohol, as well as splitting fat into these substances. This reversibility of enzymes had previously been shown for maltase by A. C. Hill; and for the ferment which synthesizes benzoic acid and glycocholic acid into hippuric acid, by Schmiedeberg, who, however, did not appreciate the significance of the reactions that he had observed. The work of Kastle and Loevenhart may be briefly summarized as follows:<sup>11</sup> Lipase will cause solutions of ethyl alcohol and butyric acid to unite to form the ester, ethyl butyrate. On the other hand, in solutions of ethyl butyrate lipase causes a splitting. In either case the end result is the same, namely, a mixture of fat, fatty acid, and alcohol; in other words, lipase simply acts to establish an equilibrium between these substances, and the effect of the enzyme is merely to hasten this equilibrium which would be attained more slowly without its aid. Subsequently it was found that all the tissues in the body that were tested for lipase showed its presence, most notably in the liver, active mammary gland, blood, lymph, and intestinal mucosa, as well as, of course, the pancreas. Of particular interest is the fact that the liver is about two times more active, weight for weight, than the pancreas of the hog, and of course in its total bulk many times more. Subcutaneous fat was found to be both lipolytic and lipogenetic, as also are the pericardial and perinephritic fat, showing the presence of lipase in adipose tissue.

The history of fat in the body may now be considered to be as follows: The lipase in the stomach does not act, because of the presence of hydrochloric acid. In the intestines lipolysis occurs, with production of a mixture of fat, fatty acid, and alcohol—usually glycerin. But, as the fatty acid and glycerin are diffusible, while the fat is not, they are separated from the fat by absorption into the wall of the intestine. Hence an equilibrium is not reached in the intestine; so the splitting continues until practically all the fat has been decomposed and the products absorbed. When this mixture of fatty acid and glycerin first enters the epithelial cells lining the intestines, there is no equilibrium, for there is no fat absorbed with them as such. Therefore the lipase, which Kastle and Loevenhart showed was present in these cells, sets about to establish equilibrium by combining them. As a result we have in the cell a

<sup>10</sup> *Chemical News*, Vol. LXXXIII (1901), Nos. 2150-2155; and also *American Chemical Journal*, Vol. XXIV (1900), p. 491.

<sup>11</sup> For a more complete discussion of fat metabolism see A. S. LOEVENHART, "On the Relation of Lipase to Fat

Metabolism—Lipogenesis," *American Journal of Physiology*, Vol. VI (1902), p. 231; also H. GIDEON WELLS, "Reversibility of Enzymes and its Application to Physiological and Pathological Processes," *Journal of the American Medical Association*, January 25, 1902.

mixture of fat, fatty acid, and glycerin, which will attain equilibrium only when new additions of the two last substances cease to enter the cell. Now another factor also enters, for on the other side of the cell is the tissue fluid, containing relatively little fatty acid and glycerin. Into this the diffusible contents of the cell will tend to pass to establish an osmotic equilibrium, which is quite independent of the chemical equilibrium. This abstraction of part of the cell contents tends to overthrow chemical equilibrium again, there now being an excess of fat in the cell. Of course, the lipase will, under this condition, reverse its action and split the fat it has just built into fatty acid and glycerin. It is evident that these processes are all going on together, and that as the composition of the contents of the intestines and of the blood vessels varies the direction of the enzyme action will also vary. In the blood serum, and also in the lymphatic fluid, there is more lipase which will unite part of the fatty acid and glycerin, and by removing them from the fluid about the cells favor osmotic diffusion from the intestinal epithelium, thus facilitating absorption.

Quite similar must be the process that takes place in the tissue cells throughout the body. In the blood serum bathing them is a mixture of fat and its constituents, probably nearly in equilibrium since lipase accompanies them. If the diffusible substances enter a cell containing lipase, *e. g.*, a liver cell, the processes of building and splitting will be quite the same as in the intestinal epithelium. The only difference is that here the fatty acid may be removed from the cell by being utilized by oxidation or some other chemical transformation.

With this explanation of the physiological processes of fat metabolism in mind, a reconsideration of the subject of fat necrosis leaves one in considerable confusion. (1) Since lipase is a normal constituent of fat tissue, how can it be that the presence of lipase of pancreatic juice is responsible for the changes of fat necrosis? (2) The splitting of fat occurring normally and constantly in the fat cells, is it possible that the products of this decomposition or the lipase itself can cause the death of the fat cells? (3) The presence of masses of free fatty acids in the fat tissue does not at all agree with the idea of reversible action of lipase. If the action of lipase is to cause a balance between the fats and their acids and alcohol, the presence of added lipase in the fat tissue should have little effect; the only one conceivable would be an increased accumulation of fat, in no wise different from that due to increased ingestion of fats. (4) Ferments are not diffusible, yet one not infrequently finds the lesion deep in the fat tissue when the lipase has simply been introduced into the peritoneal cavity. Fat necrosis not infrequently occurs as a widespread process, invading not only the entire peritoneal fat, but also the subcutaneous, pericardial, and subpleural fat; it is difficult to explain this distribution and localization of a ferment. The focal character of the lesion is equally perplexing.

All these questions and facts leave much doubt as to the genesis of fat necrosis through action of lipase, while they offer no substitute explanation. The facts of which we are sure, and upon which must be based the study of the problem, are the fol-

lowing: (1) An actual necrosis of fat tissue occurs which is sharply circumscribed; (2) within the necrotic area splitting of the fats takes place, leading to the presence of free fatty-acid crystals in large masses; (3) these lesions are present in man, only associated with escape of pancreatic juice from its normal channels, and have been produced in animals always by similar processes; (4) the etiological factor may be distributed to points remote from its original site.

The questions to be answered are: (1) Is a ferment responsible for fat necrosis, or can it be produced by agents that are not ferments? (2) If a ferment, is it lipase or one of the other ferments of the pancreatic juice or of the tissues? (3) If lipase, is it the lipase of the pancreatic juice or of the fat tissue itself that is acting? (4) Is the necrosis of the tissue primary and the splitting of the fat secondary, or, as seems to be commonly accepted, is the splitting of the fat the first step and the necrosis a result of this lipolysis? (5) How is the peculiar dissemination of fat necrosis brought about, and why is the process focal?

#### IS A FERMENT CONCERNED IN FAT NECROSIS?

It is quite remarkable that in the experiments so far performed no attempt has been made to determine this point by the usual method for detecting ferment action, that is, the susceptibility of ferments to heat. Probably this is because nearly all experiments so far have been made by causing an injured or transplanted pancreas to yield its secretion *intra vitam*, under which circumstances study of the acting fluid was not easy. To determine the actual presence and agency of a ferment, it was necessary to secure an extract that could be manipulated outside of the body, and that would produce fat necrosis with a considerable certainty. In view of Langerhans's results, but a single focus in twelve experiments, the simple preparation made by triturating fresh pancreas with water did not offer a good prospect, but, as it was the simplest, it was tried first. The results were surprising, in that the extract of fresh hog's pancreas never failed to cause a decided fat necrosis in cats, dogs, and rabbits. Probably Langerhans's poor success was due to the much slighter activity of the pancreas of the rabbit, which he used, as compared with that of the hog. Loevenhart found that the hog's pancreas gives a much more actively lipolytic extract than does that of the dog, and it is quite probable that the herbivora would have a still weaker pancreatic juice. The extract used was made as follows: 50 grams of hog's pancreas, removed about three hours before the experiment, was washed quickly in sterile water, then in 95 per cent. alcohol, and again in sterile water, to remove as many as possible of the bacteria on the surface of the gland, necessarily present from the handling at the slaughter-house. It was then minced in a sterilized meat-chopper, collected in sterile dishes, and triturated with sterile quartz sand in 100 c.c. of the solution to be used. In one series the fluid was 1 per cent. sodium carbonate, and in another 0.4 per cent. acetic acid. The gland had of itself a slight initial acidity to phenol-phthalein. After standing a short time to permit the sand to settle out, the

fluid was strained through gauze, which permitted a considerable amount of pulp to pass into the filtrate. From 10 to 50 c.c. of this fluid was then injected into the abdominal cavity of cats, dogs, and rabbits, after the various indicated manipulations had been performed.

*Protocol 2.—Dog, received intraperitoneal injection of an alkaline solution of fresh hog's pancreas. Killed after five days, and fat necrosis found.*

2-11-'02. Injected intraperitoneally 50 c.c. of emulsion of fresh hog's pancreas in 1 per cent.  $\text{Na}_2\text{CO}_3$ .

2-16-'02. Appears perfectly well, and has been so ever since the first day after the injection. Killed with chloroform. *Autopsy*: Omentum lies over the upper half of the abdominal cavity, adherent by firm hemorrhagic fibrinous adhesions to the parietal wall and to the intestines. A mass of intestines is adherent beneath the omentum, and in the coils are a few small pockets of pus. Scattered about in the omental fat are many irregular areas, pinhead size and larger, appearing somewhat more yellow than the fibrin masses. There are a few such areas in the mesentery. The areas are not all near the point of greatest inflammation, but some are in fat that appears otherwise normal. Pancreas and other viscera appear normal. *Cultures* from the abdominal fluid yielded a bacillus in morphology like *B. coli communis*. *Histologically* the areas described are seen to be typical foci of fat necrosis, with considerable leucocytic demarcation.

*Protocol 51.—Cat, received intraperitoneal injection of an alkaline solution of fresh hog's pancreas. Death after about twelve hours; typical fat necrosis.*

4-22-'02, 11:30 A. M. Injected intraperitoneally, under chloroform anaesthesia, 30 c.c. of emulsion of fresh hog's pancreas in 1 per cent.  $\text{Na}_2\text{CO}_3$ .

4-23-'02, 8 A. M. Found cold and stiff. Probably had not survived the injection more than twelve hours. *Autopsy* showed an extensive fibrinous deposit over all the peritoneal surfaces, with a considerable quantity of turbid, pinkish fluid. In the omental fat were several areas that seemed to be typical fat necrosis. Pancreas and other viscera showed no change. *Cultures* from the fluid yielded the staphylococcus pyogenes aureus, and an undetermined bacillus. *Histologically* there were found in the omentum, in addition to typical minute foci of fat necrosis, areas of more general necrosis affecting all of one side of the omentum, the other side being unaffected, a sharp line of demarcation between the necrotic and the unaffected tissue being present. There were some places where the necrosis extended entirely through the omentum.

*Protocol 56.—Cat, injected with alkaline emulsion of pancreas. Death after eighteen hours; result positive.*

4-29-'02, 1:45 P. M. Ten c.c. of a 1 per cent. solution of  $\text{Na}_2\text{CO}_3$  containing emulsion of fresh hog's pancreas injected intraperitoneally.

4-30-'02, 9 A. M. Found dead, but body still warm. About one ounce of turbid fluid found in the peritoneal cavity, the surfaces being cloudy and hyperæmic. A few foci of necrosis seen in the pro-peritoneal fat, retroperitoneally, and in the mesentery; more in the omentum, but nowhere very abundant. The largest are the size of a pinhead. There are also stripes of fat necrosis in the omentum extending along the fat trabeculae. A few foci in the pericardial fat in the lower part, but none in the fat above the heart. Pancreas and other organs show no changes. *Cultures*: an agar slant developed a profuse growth, but was not worked out. *Histologically* typical fat necrosis in foci and *en masse* as in 51.

*Protocol 50.—Dog, injected with acid extract of pancreas; death in about twelve hours; result positive.*

4-22-'02, 1:30 P. M. Injected with 50 c.c. of an acid extract of fresh hog's pancreas, made by triturating the gland substance with 0.4 per cent. acetic acid; after straining through cheese-

cloth, the fluid was allowed to stand one and three-fourths hours at room temperature, to permit the acid to have whatever effect it might upon the enzymes present.

4-23-'02, 8 A. M. Found dead and cold; could not have survived the injection more than twelve hours. *Autopsy*: peritoneal cavity contains turbid, fibrin-flecked fluid. Omentum slightly adherent over intestines and studded with clear white spots, from pin-point size to confluent areas one or more centimeters in diameter. The mass of fat attached to the anterior abdominal walls is studded with similar spots, without evidence of inflammation. Smaller numbers were also present in the retro-peritoneal, mesenteric, and pericardial fat tissue. *Cultures* all gave growth of an organism resembling, on agar slants, the growth of *B. mucosus capsulatus*, but it was not followed out. *Histological* examination shows typical fat necrosis.

*Protocol 53.*—Cat, received acid extract of pancreas, death in about twelve hours; result positive.

4-22-'02, 2:30 P. M. Injected 20 c.c. of an emulsion of fresh hog's pancreas in 0.2 per cent. acetic acid, after it had stood for about two and three-fourths hours.

4-23-'02, 2 A. M. Found dead and cold; must have died not much later than twelve hours after injection. *Autopsy*: peritoneal cavity contained much turbid fluid and fibrin. The animal was emaciated and the fat was small in amount, but in the omentum were occasional fairly typical foci, and a few others in the mesentery, but none found elsewhere. *Histologically* the fat necrosis was found to be typical, but more extensive than appeared to the naked eye, the entire surface of the omentum in considerable areas showing a necrosis for a depth of one or two cells.

*Protocol 60.*—Cat, received injection of pancreas emulsion in acid; death after eighteen hours; result positive.

4-29-'02, 4 P. M. Injected into peritoneal cavity 20 c.c. of an emulsion of fresh hog's pancreas in 0.4 per cent. acetic acid which had stood two hours.

4-30-'02, 10 A. M. Found dying and chloroformed. Typical fat necrosis, moderate in abundance, distributed generally throughout the omentum, mesentery, and retro-peritoneal fat, the largest areas being not larger than a pinhead. There were also several typical areas in the pericardial fat, but none above the heart. The contents of the peritoneal cavity were slightly acid to phenol phthalein. *Cultures* showed abundant growth of unidentified organisms. *Histological* examination shows most extensive fat necrosis, often passing entirely through the omentum in large areas, and notable for the absence of leucocytic invasion.

*Protocol 52.*—Rabbit, injected with pancreas emulsion, alkaline, that had been boiled. Inflammation, but no fat necrosis.

4-22-'02, 3 P. M. Injected with 30 c.c. of the same emulsion as No. 51, that is, in 1 per cent.  $\text{Na}_2\text{CO}_3$ , after it had been heated to boiling in the water bath for twenty minutes.

4-23-'02, 10 A. M. Seems well; killed. Abdominal cavity contains a considerable amount of fibrinous exudate, not greatly different in amount and nature from that seen in the animals injected with unboiled extract, but no traces of fat necrosis. *Cultures* remained sterile. *Histological* examination of the omentum, which was preserved entire and examined throughout, showed no fat necrosis, merely acute inflammation.

*Protocol 58.*—Cat, injected with alkaline pancreas emulsion that had been heated to boiling. Killed after three days; result negative.

4-29-'02. Injected 20 c.c. of pancreas emulsion in 1 per cent.  $\text{Na}_2\text{CO}_3$  that had been heated 10 minutes at  $100^\circ\text{C}$ .

5-2-'02. Has been quite well. Killed by chloroform. Peritoneal cavity shows a few threads of fibrin, otherwise no evidence of inflammation, and no signs of fat necrosis. *Microscopic* examination showed a slight leucocytic infiltration, but no fat necrosis.

*Protocol 61.*—Cat, received alkaline pancreas emulsion that had been heated to boiling. Killed at the end of three days. Result negative. A duplicate of No. 58.

It will be seen that in not a single case did the unboiled hog's pancreas, whether in alkaline or acid solution, fail to produce a distinct fat necrosis, while the injections of similar preparations that had been boiled failed to cause any such process, macro- or microscopically. It is thus certain that in the fresh hog's pancreas there is some substance that is destroyed by boiling, and without which the pancreatic extract is unable to cause fat necrosis. The chief criticism of these experiments is that bacteria were not excluded—there were culturable organisms in the peritoneum of all the animals with fat necrosis, while cultures from the animals injected with boiled extract were sterile, showing that the bacteria were present in the pancreatic extract as prepared. The treatment necessary to secure an active emulsion of pancreas renders it difficult, if not impossible, to maintain sterility, but fortunately it was found not to be necessary to obtain pancreas extract in this way to produce fat necrosis. It was found that the ordinary commercial "pancreatin" prepared by various firms was invariably effective, even although the preparation used was several months old.<sup>12</sup> There was never a failure to produce fat necrosis by injecting intra-abdominally into a cat or dog as much as 10 c.c. of a 5 per cent. emulsion of this preparation, and often the process was very extensive, even more so than any described from the operative methods used by various experimenters. It made no difference whether the solution was alkaline, acid, or neutral, as with fresh pancreas. Experiments made to ascertain the minimum amount of pancreatin that would cause fat necrosis showed that amounts as small as 0.05 gm. of commercial pancreatin might sometimes produce a few foci, and again sometimes fail entirely.<sup>13</sup>

Because of its simplicity and effectiveness this method can be recommended for teaching purposes, as it gives a ready method for demonstration of the appearances of fresh fat necrosis as seen by the surgeon. The frequency with which surgeons mistake this condition for miliary tuberculosis leads one, in fact, to urge that this demonstration be frequently made.

Pancreatin seems to be free from pathogenic bacteria, probably because of its dry condition; at least cultures from animals with fat necrosis produced by this preparation were quite generally sterile. This, therefore, excludes bacteria as a causative agent in these experiments, an exclusion not absolutely essential, since many investigations have already shown the non-bacterial nature of fat necrosis. Marx<sup>14</sup> reviews the literature of this subject and finds that in human beings the results have been variable, colon bacilli being often found, and sometimes cocci, especially when accompanying marked pancreatitis. The very variability of these results is in favor of the accidental nature of the presence of bacteria. Further, in acute peritoneal infections of other than pancreatic origin fat necrosis is not found. In experimental fat necrosis Opie found that pieces of necrotic fat were sterile.

<sup>12</sup> Preparations put out by the following firms were tried, and all gave positive results: Armour & Co.; Truax, Greene & Co.; Parke, Davis & Co.; Fairchild Bros. & Foster; and Merck (not the scale preparation).

<sup>13</sup> In these, as in all subsequent experiments, unless

otherwise specified, the preparation of Armour & Co. was used, not because of any particular activity, but because it was first used and it seemed desirable to use the same preparation throughout to make the results comparable.

<sup>14</sup> Virchow's *Archiv*, Vol. CLXV (1901), p. 290.

As with the fresh pancreatic extract, boiling was found to prevent the production of fat necrosis, without fail, no matter what the reaction of the fluid. It now remained to ascertain the temperature at which this property was destroyed.

#### THERMAL DEATH POINT

A series of experiments was performed, in which 1 gm. pancreatin in 20 c.c. distilled water was kept for a period of 5 minutes at temperatures within a narrow range; then, after cooling, 10 c.c. containing about 0.5 gm. was injected intraperitoneally. Abstracts of the protocols follow:

*Protocol 79.*—Medium-sized dog, injected intraperitoneally with 10 c.c. of emulsion of distilled water and Armour's pancreatin, 0.5 gm., that had been heated five minutes between 55° and 60° C. After three days it was killed and abundant and typical foci were found in the omentum and in the fold of the anterior peritoneum.

*Protocol 82.*—Cat, received intraperitoneally 0.5 gm. pancreatin in water that had been heated to 70°, and then slowly cooled to 60° during fifteen minutes. Killed after two days. At one point in the omentum were found a few minute white points, grouped together, which microscopic examination showed to be areas of fat necrosis, with considerable infiltration with leucocytes.

*Protocol 81.*—Dog, received emulsion of pancreatin that had been heated for five minutes at from 68° to 70°. Killed after two days, and in the omentum are perhaps a dozen minute, pin-point-sized spots which are seen microscopically to be foci of fat necrosis, marked by intense invasion of leucocytes.

*Protocol 87.*—Twenty c.c. of water containing about 1 gm. of pancreatin was poured through an abdominal wound in a cat, upon which had been performed a laparotomy for the purpose of removing part of the omentum, in connection with another experiment. The mixture had been heated for about five minutes at from 65° to 71°. Killed after two days, and about the site of the ligature on the omentum the fat was found streaked with white, which in some places formed considerable areas of changed fat. More remotely in the omentum and in the mesentery were a few very minute white spots. All these white portions of the fat were found under the microscope to be necrosed fat.

*Protocol 107.*—A cat received 10 c.c. of emulsion of pancreatin that had been heated to from 71° to 74° for five minutes. Killed after two days and no traces of fat necrosis could be found.

*Protocol 91.*—Small dog, received an injection of 10 c.c. of pancreatin emulsion that had been heated during five minutes at between 75° and 79°. Killed after two days, and no fat necrosis could be found.

*Protocol 94.*—Through a laparotomy wound in a small dog was poured 15 c.c. of an emulsion containing 0.5 gm. pancreatin, which had been heated at from 80° to 81° for four minutes. Killed after two days and no fat necrosis could be found.

As will be seen, at temperatures not over 60° the pancreatin is still quite active, but less so than when unheated. Two experiments at temperatures reaching 70°, but staying for the greater part of the time a few degrees lower, gave a few foci that were characterized by early invasion of leucocytes and healing. A third preparation of the same temperature used in an animal that had suffered an injury to the omentum immediately before caused a much more marked necrosis, chiefly about the site of the

injury, where the action of the agent seemed to have been favored. Temperature above  $71^{\circ}$  invariably destroyed the property of the pancreatin to cause fat necrosis. Probably the death point of the active agent is not a fixed one, but will vary within slight limits in different preparations and in solutions of different reactions. This point has not as yet been investigated farther.

The agent contained in extracts of fresh hog's pancreas and in dry commercial pancreatins is, therefore, destroyed by heat; in the case of the latter the maximum temperature that can be withstood for five minutes in an aqueous solution is not above  $71^{\circ}$ . This observation seems to indicate that we have here to deal with a ferment, almost certainly, one is tempted to say, for the best-known ferments suffer loss of activity at just about such temperatures. The close agreement of the experiments pointing to a reduction of activity at temperatures from  $65^{\circ}$  to  $71^{\circ}$ , with entire loss of power above this point, offers a hopeful field for study to ascertain the exact nature of the ferment, if it is a ferment, that causes fat necrosis. Experiments on this feature are being performed, and interesting results have been obtained, which will be published in a subsequent paper, on their completion.

#### CAN SUBSTANCES OTHER THAN FERMENTS CAUSE TRUE FAT NECROSIS?

Since the idea of enzyme reversibility did not agree with the facts known concerning fat necrosis, more particularly the presence of large amounts of fatty acids in the cells where normally fat exists, it seemed possible that some substance that interfered with equilibrium might be present, accounting for the continued splitting without synthesis. The pancreatic juice, in addition to the ferments, contains a considerable amount of alkali carbonate. Loevenhart has found that lipase cannot synthesize the ester from sodium butyrate and ethyl alcohol, and on investigation of the literature he found much evidence that in normal metabolism the fatty acids do not form soaps. Munck has showed that soaps are highly toxic; sodium oleate, although less toxic than the palmitates and stearates, caused death of rabbits in doses of 0.13 mg. per kilo when injected intravenously. On the other hand, free oleic acid is not toxic. Pflüger has shown that 1 per cent.  $\text{Na}_2\text{CO}_3$  saponifies the higher fatty acids very slowly and incompletely at  $37^{\circ}$ , so that presumably there is but little chance of such a combination occurring in the body in a fluid of the alkalinity of the blood. However, it was quite natural to suspect that a solution so strong as the pancreatic fluid might cause a union of alkali with fatty acid when freshly split, as in living fat. If this occurred, since the soap cannot be synthesized into fat by lipase, it would be impossible for equilibrium to be attained, and splitting would go on until no fat remained. This would at least account for the removal of the fat from the necrotic area, although it would not explain the presence of crystals of free fatty acids observed in the necrotic cells. Accordingly the effect of alkaline solutions upon the fat tissues was tried. One per cent. and 2 per cent.  $\text{Na}_2\text{CO}_3$  solution, sterilized in the autoclave, when injected intraperitoneally caused nothing at all comparable to fat necrosis,



although it did produce a considerable degree of acute inflammation without bacterial aid. Pancreatic extract and pancreatin did not cause fat necrosis when boiled in a solution of 1 per cent.  $\text{Na}_2\text{CO}_3$  and injected with it, as mentioned previously, and weak acetic acid solutions were equally inert. Injection of 2 per cent.  $\text{Na}_2\text{CO}_3$  directly into the subcutaneous fat of dogs caused no necrosis, but a diffuse hemorrhagic extravasation with oedema and leucocytic invasion. One per cent.  $\text{Na}_2\text{CO}_3$  containing inactive pancreatin, injected into subcutaneous fat, caused an extensive inflammation with great leucocytic infiltration, but without any necrosis of tissue and nothing at all resembling fat necrosis. Strong solutions of  $\text{NaOH}$  naturally caused much greater changes. Injection of 1 c.c. of a 10 per cent. solution subcutaneously in the tissues of a dog produced a large area of gangrene very quickly; in one hour the area was well defined, of a dark red color, with sharp borders, and on section the subcutaneous tissue was hemorrhagic and softened. Microscopically stained specimens showed that there is no sharp line of demarcation between the dead and living tissue. The fat suffers just the same as the tissue in which it lies. After twenty-four hours the skin is black and soft, but still intact; beneath it is undermined by liquefaction of the subcutaneous tissue, replacing which is an oily, purulent-appearing fluid. This fluid contains so many red cells after cutting into it that it cannot be well examined until cleared up with weak acetic acid. Then it is seen to consist largely of fat droplets of varying size, in and about which are many crystals of radiating clusters of needles, each individual crystal being shaped like an icicle. The crystals are found only where the red cells are being dissolved by acetic acid, and perhaps are the result of the action of acid on soaps. Sections taken from the edge of the zone of necrosis after seventy-two hours show in hardened and stained preparations irregular areas consisting of a group of fat cells full of a solid, eosin-staining substance. In every respect these areas resemble fat necrosis, except that they are not in as regular groups and are not so well demarcated. Leucocytes that appear normal are seen within the necrotic areas, and occasionally a line of leucocytes resembling the line demarcating areas of true fat necrosis can be found. Such pictures, which are infrequent, offer no distinct differences from the early stages of fat necrosis.

The possibility that the formation of calcium soaps played an important part by precipitating out fatty acids and destroying the equilibrium suggested that perhaps an alkaline calcium solution might cause conditions resembling fat necrosis. Subcutaneous injections of saturated solution of  $\text{Ca}(\text{OH})_2$  caused merely an acute inflammatory reaction (the solution was not sterilized), without alterations, in the fat tissue, and without formation of any discernible accumulation of insoluble calcium salts after twenty-four hours.

As fat tissue contains lipase, the question arose as to the possibility of it by itself causing the changes in the fat. To study this, portions of the omentum of dogs were shut completely out of all relation to the surrounding fluids and tissues by inserting a part of the omentum in a sterilized rubber finger-cot, and then deprived of nutrition

by a ligature tied tightly about the base of the cot. In this way necrosis was produced in the fat, presumably without injury to any enzyme it might contain, without disturbing its chemical equilibrium, and without the influence of outside factors. Within three days the pieces sloughed off at the point of ligation and were found loose in the abdominal cavity, containing a soft and brownish fat. Sections of this fat showed it to be totally necrotic, with opaque, thick fibrils of connective tissue, and with but a few abnormal nuclei surviving. The fat cells were completely empty in stained and hardened sections, and there was no resemblance whatever to fat necrosis. Frozen sections stained by sudan III showed that the fat was still present in the fat cells, staining well, apparently in a perfectly unchanged condition.

The part played by fatty acids in the necrosis was studied by placing lumps of sterilized palmitic acid and stearic acid in the subcutaneous fat tissue, and in a fold of the omentum in contact with fat tissue, where it was retained by a suture that formed a pocket. After two and four days the pieces with the surrounding tissue were taken out and hardened in formalin. Microscopic examination showed that the lumps of fatty acids had acted like perfectly inert foreign bodies, causing no changes but proliferation and a slight leucocytic infiltration of the tissues immediately adjacent.

#### ACTION OF PANCREATIN POST MORTEM AND IN VITRO

Not infrequently at autopsy necrosis of the fat immediately about the pancreas, and more particularly the fat lying between the lobules of the gland, has been observed (Chiari).<sup>15</sup> At times cellular reaction has established the vital nature of the fat necrosis, but in other cases the necrosis is more diffuse and without cellular reaction, suggesting a change in the fat of *post-mortem* origin, quite analogous to the common self-digestion of the gland. After death the digestion of the gland destroys its consistence to such an extent as readily to permit of the escape of its fluids beyond its confines, and the question of the possibility of this pancreatic fluid producing fat necrosis after death becomes of interest. If fat necrosis is simply the reaction of fat tissue to certain enzymes, it is reasonable to expect pancreatic extracts to cause the process after death, or even *in vitro*, if the temperature is kept favorable for enzyme action. The following experiments were performed:

#### EXPERIMENTS IN VITRO

Pieces of fat omentum were placed in a 1 per cent.  $\text{Na}_2\text{CO}_3$  solution containing pancreatin, and kept at body temperature in the incubator. At intervals portions were placed in formalin and examined histologically, both in frozen and imbedded sections. It was found that within half an hour the cells on the surface had lost their staining entirely, and the membrane surrounding the fat cells had assumed an increased affinity for eosin and had become thickened. The appearance was exactly similar to the change seen in fat tissue in the earliest stages of fat necrosis (see subsequent description) before the necrosed fat cells become filled with the characteristic opaque material. The changes that follow from longer exposure simply consist of a loss of nuclear stain throughout the fat tissue, and later an entire loss of even the connective tissue structure.

<sup>15</sup> *Zeitschrift für Heilkunde*, Vol. XVIII (1896).

## POST-MORTEM EXPERIMENTS

*Protocol 75.*—Injected 20 c.c. of 1 per cent.  $\text{Na}_2\text{CO}_3$  solution containing 1 gm. of pancreatin intraperitoneally in a dog, immediately after it had been chloroformed to death. The body was left at room temperature over night. At autopsy there were found throughout the peritoneal fat many groups of small ecchymotic spots, about some of which were minute pale areas, barely visible, that suggested fat necrosis, but they were too small and indistinct to be positively identified as such. Sections of the omentum (in all cases in this work the tissues were hardened in formalin and imbedded in celloidin or frozen) show areas in which the fat tissue has lost its staining properties. Some of the fat cells in these areas contain finely granular material, more are empty; never is the amount comparable to that seen in the fat cells of fat necrosis. Occasionally the contents take the eosin stain, but never the hæmatoxylin. The areas are at the margins of the fat, and in size and outline are much like foci of fat necrosis. In fact, the chief difference observable is in the small amount of granular material in the fat cells, absence of any blue stain, and absence of leucocytes.

*Protocol 85.*—Dry pancreatin was sprinkled upon the anterior fold of fat tissue in the abdomen of a dog that had just been killed, and the fat tissue was folded over it. The body was sewed up and kept at room temperature for seventeen hours. *Autopsy:* At the place where the pancreatin was placed there was a soft, cheesy mass of fat toward the surface; deeper in there were minute, barely visible opacities in the fat, too small to be identified. Sections showed that much of the fat, particularly on the surface, had lost its nuclear staining, and the fat cells contained a granular material which in some instances stained bluish, in other with eosin. In places this change is seen extending in a short distance from the surface, resembling fat necrosis strikingly. As a matter of fact, there is no essential difference between these changes and true fat necrosis. The differences are of degree rather than of kind.

*Protocol 117.*—Wads of cotton saturated with an emulsion of pancreatin were placed in the omentum, and held in place by sutures of silk, tied loosely to prevent interference with circulation. The dog was kept alive under anaesthesia for one hour, and pieces were cut out at the end of one-half hour and one hour. The dog then died, and the body was left at room temperature for eighteen hours. Sections of the tissue at one-half hour showed merely death of the peritoneum and some of the superficial fat cells, none of which contained any solid intracellular material. At one hour the necrosis had extended deeper into the fat tissue, without solidification of the contents of the fat cells. The sections of tissue removed after the body had been dead for eighteen hours showed that the outer layers of fat cells were necrotic, as in the other specimens; all of these and many deeper ones that were not necrotic were full of a solid exudate. It looked no different from the *intra-vitam* reaction, except that the process seemed to have extended somewhat since death with the development of the characteristic solid cell contents.

*Protocol 78.*—Dry pancreatin was dusted over the omentum of a cat that had just been laparotomized for another purpose. Five hours later it was found that the abdominal wound, which had been sutured, had ruptured and the omentum was strangulated through the opening, apparently for some time, as it looked gangrenous. In some places in the omentum where it was not gangrenous were what appeared to be foci of necrosis, still very small and indistinct. Sections through the gangrenous part showed the entire surface to be necrotic, while the deeper cells appeared normal. It resembled ordinary necrosis of fat except that the surface cells were full of granular material resembling exactly the solid cell contents of fat necrosis. In other words, this tissue shows a mixture of simple necrosis of fat, and true "fat necrosis" which might be expected because of the conditions present, namely, gangrene and pancreatin. The sections look exactly like fat necrosis, except in the diffuseness of the process and the absence

of limitation or inflammatory reaction; on the other hand, it looks exactly like the sections of *in-vitro* digestion, except in the solid material that fills some of the superficial cells. It illustrates well the relationship of these processes.

These results seem to indicate that each of the processes that occur within the fat cells in fat necrosis may be produced experimentally outside the body, or within the body after death, with pancreatin. The typical anatomical picture is not reproduced, however, either macroscopically or minutely. The difference is always essentially one of degree rather than kind, suggesting that the characteristic grouping depends upon certain factors of circulation, or other processes inseparable from life.

#### STUDY OF THE SUCCESSION OF CHANGES DURING THE DEVELOPMENT OF FAT NECROSIS

Previous experimental work, depending generally upon the extravasation of the secretion of the pancreas, has given little opportunity for study of the order of events that take place in the production of the typical foci of necrosis. The method of using pancreatin solution makes this very simple, and such study is of great interest. A number of experiments were performed as follows: A dog was anæsthetized and the abdominal cavity opened aseptically; the omentum was drawn out, and at points separated as widely as possible small wads of cotton, saturated with a 5-10 per cent. suspension of pancreatin in distilled water, were placed within a fold of the omentum, and retained there by a suture, tied loosely to prevent disturbances of circulation. The omentum was then returned to the abdominal cavity, and the abdominal walls were united by heavy through-and-through sutures, held together with artery forceps instead of by tying. The animal was kept anæsthetized for the duration of the experiment, and at stated intervals the sutures were loosened, the omentum brought out, and a piece containing one of the cotton wads ligated off and removed, after which the omentum was returned to the abdominal cavity. The portions of fat in the removed piece of omentum that had been in contact with the cotton were placed in formalin for study. Sometimes the animal was permitted to come out of the anæsthetic after a few hours, and killed twenty-four hours later to study the later changes, but generally it was chloroformed to death after five or six hours. Several such experiments were performed, and the results were practically the same in all, the differences being but trivial and chiefly quantitative. From them the manner of development of the typical focus of fat necrosis was found to be about as follows:

*Fifteen minutes.*—The changes at this time consist of a general engorgement of the capillaries between the fat cells, with, at places, hemorrhages. (The amount of hemorrhage varies greatly in different cases, and as it is much more abundant in these experiments than in simple injection experiments, probably much of it is due to the unavoidable manipulation of the omentum.) The peritoneal endothelium is swollen, and in many places necrotic, as shown by a loss of visible nuclei while the cytoplasm becomes granular and often disintegrated. Where the endothelium is destroyed the underlying connective tissue is frequently swollen, the fibrillar outlines obscured, and the eosin staining increased in intensity.

*Thirty minutes.*—The changes noted at fifteen minutes have become more extensive, and in a few places the fat cells nearest the affected surface have lost their nuclei and have undergone changes in their walls indicating necrosis. There is a slight leucocytic infiltration beginning throughout the fat tissue. The necrotic fat cells are entirely empty in hardened sections. In frozen sections they contain what appears to be normal fat, staining by sudan III, and without crystals.

*One hour.*—Many of the small vessels contain hyalin thrombi, and the infiltration of leucocytes has become more extensive. There are more necrotic cells than at thirty minutes, but they still contain normal-appearing fat, without crystals. Where the areas of hemorrhage are near the surface, when the surface is necrotic, the red cells have lost their outline and appear as a homogeneous yellow mass.

*Two hours.*—The changes have increased only in degree, with the exceptions that the leucocytes have to a considerable extent migrated to the margins of the areas of necrosis, and in some places form a wall about part of the area.

*Three hours.*—By this time the accumulation of the leucocytes at the boundaries of the necrosed tissue have become more dense, and many of the areas are well walled off, so that the resemblance to typical fat necrosis is readily seen. About this time is first seen the presence of an eosin-staining material in a few of the most superficial fat cells, which has not been dissolved out by the alcohol and ether used in imbedding in celloidin. Some of the cells are completely filled with this substance, while others are but partly so; no crystals can be found in this solid substance. In frozen sections the contents of these superficial cells are opaque, structureless, and no crystals are present; when stained by sudan III this material stains a faint yellow, in some cells not at all, with a few interspersed fat granules that stain deeply. The cells farther from the surface contain fat that stains well. Areas of hemorrhage, which are marked in some instances and slight in others, show considerable disintegration of the erythrocytes with formation of pigment, especially when the tissue between the blood and the surface is necrotic.

*Four hours.*—There are no essential differences between the condition at this time and at three hours, except that, because of more extensive solidification of the contents of the fat cells and more demarcation by leucocytes, the picture of true fat necrosis is often completely produced. I have never been able to find crystals as early as this. In some four-hour specimens can be found fat cells located at the margin of the encompassing wall of leucocytes in which solidification has taken place, where the solid material takes a blue stain with hæmatoxylin about the same in intensity as ordinary nuclear staining. The blue staining substance seems to form in fume-like projections from the wall of the fat cell, often inclosing clear spaces in the meshes it forms. This seems to mark the earliest stage in the formation of calcium salts, and from its location at the edge of the necroses it seems due to union with calcium salts diffusing in from the outside.

*Five hours.*—By this time the necrosis is extensive, and most of the fat cells that have been in immediate contact with the cotton are necrosed for a depth of from one to three rows, often deeper in places. The various stages described are merely the maximum advance for that length of time; the slighter degrees are also present in the same specimen at the same time. Occasionally single foci, consisting of but one or two fat cells that are necrotic and walled in by leucocytes, are seen deep in the tissue, and, as shown by serial section, in no way connected with the surface; sometimes these isolated areas have no evident relation to the vascular structures, but not infrequently they are on the margins of large lymph spaces which are usually widely dilated during this inflammatory process. Rarely at this time are found fatty-acid crystals in frozen sections, and still more rarely in celloidin preparations. After six hours they generally become quite abundant. When they are present in frozen sections they can usually be found in the celloidin preparations, but always in much smaller numbers.

From the sixth hour on the changes vary greatly in different cases, depending on factors to be discussed later, but by this time the production of true and typical fat necrosis has been accomplished. To summarize these studies the changes in the evolution of focal fat necrosis are as follows:

First the structures in immediate contact with the pancreatin suffer, and necrosis of the peritoneal endothelium and the underlying connective tissue may occur in a very few minutes. The process seems to extend by contiguity into the fat cells nearest, and they die as did the peritoneal connective tissue. At the same time the reaction of acute inflammation goes on, and leucocytes migrate from the vessels, both deep and superficial. They tend to accumulate about the areas of necrosis, and by the end of three hours there is usually a distinct walling off. About this time the contents of the fat cells that are nearest the surface whence the process is extending undergo a change whereby their contents become solidified, the resulting substance being so little soluble in cold alcohol and ether that it passes through the ordinary process of celloidin imbedding; this substance does not stain by sudan III, and it seems entirely to replace the fat, so that when the process has filled the cell there can be found at the most but a few minute fat granules in the affected cell. Crystals that can be recognized as fatty acids have been found first about the fifth hour, but they usually are not abundant until a few hours later; they also escape solution in alcohol and ether, at least in part, and they seem to replace the solid, non-crystalline substance that first replaces the fat. After the fourth hour a basic-staining substance begins to appear in some of the cells that are solidified and nearest the still intact portions of tissue.

The subsequent changes vary greatly. In ordinary cases the amount of necrosis does not increase much after about the eighth hour, and it may stop even earlier. What will follow after this will depend entirely upon the conditions that exist. If a large amount of very active pancreatin is brought into the peritoneal cavity, the areas of necrosis will be much larger than when the amount is smaller, and the healing will take place with corresponding slowness. It is often possible to find healing as far advanced after forty-eight to seventy-two hours in some instances as it is after eight to ten days in others. The process seems to be as follows: After the area of necrosis has been fairly well walled off by leucocytes, further extension does not occur, and the process from that time on is essentially a healing one. The leucocytes nearest the surface suffer much karyorhexis, and often large masses of nuclear detritus are formed within and between the fat cells. The solid material that fills the fat cells seems to be fatty acids that have not crystallized. This is based upon the fact that they do not stain with sudan III, but replace the fat without observable intermediary steps or infiltration from outside, and that later they seem to be directly replaced by the crystals whose structure shows them to be fatty acids. One observation that is difficult to explain, particularly on this basis, is that the solid fat cells are larger than those about them and appear to be swollen; this is possibly due to the looser formation of the fatty acids. Calcification, if the presence of the blue-staining material is to be so inter-

puted, proceeding as it does from the intact tissues, is more complete and earlier in small areas. In minute foci all the cells may be full of the blue-staining material within twenty-four hours, while with large areas this substance may be found only about the edges, and never extends into the center of the foci. The amount of leucocytic invasion varies—when the foci are small the infiltration is usually great and rapid, and anything that increases the irritation, as handling or infection, seems to increase their numbers. Within twenty-four hours the small foci may be so filled with leucocytes that almost nothing can be seen of the cell contents which have been replaced by leucocytes and their débris; oftener it requires forty-eight to ninety-six hours to produce this condition. By the end of forty-eight hours there is a noticeable proliferation of new connective tissue cells at the margin of the foci, and this varies at first inversely with the amount of leucocytic invasion; with the large foci healing is chiefly by proliferation. These new cells are large, spindle, or oval, and when they have formed a considerable band about the focus of necrosis the leucocytes are found chiefly within it, apparently accomplishing absorption of the dead fat cells and their contents. (It was this zone of new connective tissue that Balser thought represented new-formed fat cells, and to which he attributed the production of necrosis through their shutting off the blood supply.) Giant cells were never seen. Occasionally leucocytes were seen partly surrounding the ends of crystals, resembling phagocytosis, and phagocytosis of nuclear fragments was frequent. Eventually the tissue is entirely replaced by the new connective tissue, containing new vessels, and the peritoneum heals over with endothelium, rarely forming adhesions. Fibrinous exudate is not usually present on the surface of areas of necrosis unless there is infection.

The areas of hemorrhage bear no particular relation to fat necrosis; sometimes they become necrotic and sometimes not. When they do become necrotic, the red blood-corpuscles soon lose their form, and a diffuse yellow mass results, with some pigment. Within three or four days the only trace left of blood is a great amount of yellow granules of pigment in areas which otherwise resemble typical fat necrosis.

The nuclear changes that occur in the cells that become necrosed are chiefly those of karyolysis, but it is quite different with the invading leucocytes, which become fragmented, often to a great extent. The small spherical granules that are left take a very intense stain, almost black, suggesting the possibility that the trypsin has split off the proteid portion of the nucleo-proteid, leaving the densely-staining nucleic acid by itself.

#### DURATION OF FAT NECROSIS

The length of time that the foci exist visible to the naked eye within the abdominal fat will evidently vary greatly with the size of the lesions. When they are small they disappear very quickly, as is shown by the following experiment:

*Protocol 80.*—7-1-'02. Injected into the abdominal cavity of a dog 10 c.c. of water containing 0.5 gm. of a preparation of pancreatin that was not very active. After the first day the dog seemed well.

7-5-'02. Opened the abdomen aseptically, under chloroform anæsthesia. In the fat of the fold of peritoneum that lies in the anterior median line of the dog's abdominal cavity were found perhaps a dozen typical foci of fat necrosis, none larger than half the size of a pinhead. A portion of the fat tissue was ligated off and removed for study. It showed typical foci, partly replaced by young connective tissue, and invaded throughout by leucocytes.

7-12-'02. Killed by chloroform. Absolutely no trace of fat necrosis could be found. A piece of tissue removed from the proximal side of the ligature, where there had been areas of necrosis seven days before, and examined histologically, showed a number of small foci of hyalin connective tissue, containing many small blood vessels and numerous large connective tissue cells.

In this case healing had been so complete in eleven days that nothing was visible to the naked eye, but the original foci had been minute. On the other hand, when large areas are produced the healing changes may not be very far advanced in eight or ten days, and it would probably be several weeks before they would be invisible, if ever. There are many cases in human beings, where foci were still abundant, in which death has occurred at much greater intervals after an operation at which the fat necrosis was detected, than in the experimental animals. However, in these cases the pancreatic juice was escaping more or less continuously, and the lesions found at autopsy may have been formed later than those seen at operation.

Fat necrosis, *per se*, seems to be of no great moment as regards the life of the subject. It is not the fat necrosis that causes the symptoms or the fatality, but the lesions of the pancreas that produced the fat necrosis, or the resulting shock, peritonitis, or gangrene. Fat necrosis is merely one of the results of escape of pancreatic juice from the gland, and means nothing more to the surgeon than that such escape has occurred. As has been observed by surgeons, a patient may recover from extensive fat necrosis without anything having been done to the pancreas itself. An animal may have extensive fat necrosis and yet appear perfectly well.

#### TIME OF APPEARANCE OF FAT NECROSIS

A few human cases have been observed in which it was possible to note that extensive fat necrosis could be produced within a comparatively few hours. H. Marx<sup>16</sup> reports a case in which laparotomy was performed upon a case of hemorrhagic pancreatitis, and no traces of fat necrosis found. Death occurred twenty-seven hours after the operation, and at autopsy, six hours after death, there was abundant fat necrosis, "areas the size of a lentil" being found. Marx also quotes a case reported by Simmons in which similar findings were made at operation and at autopsy, thirty-six hours apart. A few experiments were made upon animals to determine the minimum time. Since experiments showed that changes were visible microscopically as early as fifteen minutes after application of pancreatin and distinctly recognizable fat necrosis was present at the end of three hours, it was merely a question of how early the changes are in sufficient amount to be visible to the naked eye as the typical sub-endothelial white spots. The experiments made with wads of cotton could not be used for this purpose because there was so much hemorrhage and hyperæmia as to obscure the early changes, besides

<sup>16</sup> *Virchow's Archiv*, Vol. CLXV (1901), p. 290.



not being at all comparable to the ordinary conditions of fat necrosis. However, even here there could be seen at the end of four hours a number of minute white streaks that were highly suggestive of fat necrosis.

One experiment consisted of placing a large lump of cotton saturated with 5 per cent. emulsion of pancreatin upon the omentum, and examining the omentum under it at frequent intervals, the animal being anaesthetized. In something less than three hours and twenty minutes a few very small foci could be seen. Of more value was the following experiment: Ten c.c. of a 1 per cent.  $\text{Na}_2\text{CO}_3$  solution containing 0.5 gm. of pancreatin was injected into a dog. After five and a half hours it was killed with chloroform, and abundant small foci were present in the omentum opposite the point of injection, and a few small areas in the mesentery. Histologically there was found abundant typical fat necrosis.

In another dog, that died somewhere about twelve hours after injection, very extensive necrosis was present throughout all the abdominal fat, and even in the pericardium.

It would seem that the areas become visible to the naked eye when the solidification of the contents of the foci renders them opaque and white, which is generally about four hours after the pancreatin comes in contact with the tissues.

#### MANNER OF EXTENSION

Fat necrosis is not only found upon the surface of fat tissue lying in contact with the peritoneum, but the areas may be found deep within the intra-abdominal fat tissue; furthermore, they may be found outside the abdominal cavity, in the pericardial and mediastinal fat, and in the subcutaneous tissue. This has been observed in the human cases and also in the domestic animals. As it is difficult to understand the relation of the escape of pancreatic juice to such remote lesions, some have thought that this remote process may be of a different nature. E. L. Opie<sup>17</sup> found, however, that equally widespread fat necrosis could be produced experimentally in cats after the pancreatic ducts had been ligated for twenty days or more. As in human cases, the distribution was chiefly in the pericardium and subcutaneous fat, but foci were also observed along the carotid and subclavian arteries. Animals that survived but a few days did not show fat necrosis outside of the abdomen, except one animal which received pilocarpin after the ducts had been ligated, in order to increase the pancreatic secretion, and which showed foci in the pericardial fat four days later. Opie hardly explains the way in which the agent causing fat necrosis reaches the pericardial fat, and the fat about the great vessels in the neck. He observed that the process occurred only in animals whose pancreatic duct had been ligated for long periods, and that the foci in the pericardium showed a much younger state than those in the omentum. Also the process was less extensive more remote from the pancreas. Hence he speaks of the distant parts as being perhaps "reached by gradual diffusion through continuous layers of connective tissue." With this supposition I can hardly agree. In the first place, the

<sup>17</sup> *Contributions to the Science of Medicine by Pupils of William H. Welch*, 1900, p. 859; also *Johns Hopkins Hospital Reports*, Vol. IX (1900), p. 859.

intra-thoracic and subcutaneous foci, however numerous, are separated by considerable amounts of fat tissue that is intact. Again, I have often observed small foci deep in the omental fat, entirely separated from the surface by fat microscopically normal. Neither is any great length of time required to produce extra-abdominal foci, as is shown by the experiments of the protocols of which the following are brief abstracts:

*Protocol 50.*—Dog, received 50 c.c. of an emulsion of fresh hog's pancreas in 0.2 per cent. acetic acid intraperitoneally. Death occurred about twelve hours later. Foci of fat necrosis abundant throughout the abdominal fat, and in the lower part of the pericardial fat were a few small areas.

*Protocol 56.*—A cat, which received intraperitoneally 10 c.c. of emulsion of fresh hog's pancreas in 1 per cent.  $\text{Na}_2\text{CO}_3$ , died after about eighteen hours. Moderate amount of fat necrosis in the abdominal fat, and in the lower part of the pericardium a few foci were found, but there were none above the heart.

*Protocol 60.*—A cat received intraperitoneally 20 c.c. of an emulsion of fresh hog's pancreas in 0.4 per cent. acetic acid. Killed after eighteen hours. Fat necrosis was distributed throughout the abdominal fat in moderate amount, and a few foci were found in the lower part of the pericardium.

*Protocol 101.*—A small cat received intraperitoneally 10 c.c. of an emulsion containing 0.5 c.c. of pancreatin (Truax, Greene & Co.) in water. Killed twenty-four hours later, and an enormous amount of fat necrosis was found in the abdomen, in addition to which abundant foci were found all over the pericardium and in the fat above it as high as the clavicles, along which were a few spots. There were no foci along the ribs, but there were a few in the posterior mediastinal fat.

*Protocol 92.*—Injected into a dog 10 c.c. of water containing 0.5 gm. pancreatin. Killed after two days. In addition to a very extensive fat necrosis throughout the abdominal cavity, there were a few foci in the pericardium, but none elsewhere.

*Protocol 77.*—A large cat received 5 c.c. of water containing 0.25 gm. of pancreatin. Killed after nine days. Most extensive fat necrosis throughout the abdomen, many typical foci in the subcutaneous fat from the costal arch to the umbilicus, several large foci on the pericardium as high as the base of the heart, but none above that point. Extending along the ribs, beneath the pleura on each side, are rows of small foci, most abundant about the middle of each thoracic wall, although there are many as far back as the costo-vertebral junction.

If one considers the distribution of these lesions, especially as seen in 77 and 101, and in the human case described by Chiari, and in the experiments of Opie himself, the probability that the path of dissemination is the lymphatics is strong. In what other way could such a localization occur without involving all the intervening tissue? As will be noticed, all the foci are found along the course of the lymphatic channels, particularly the beadings between the ribs, along the course of the intercostal vessels. In just such a way is miliary tuberculosis spread, with the bacilli localizing their action at various points along the lymph vessels, favored by local stagnation or obstruction. In favor of this view is the following observation: In the fat below the surface of the omentum in the experiments performed to show the sequence of changes, there were frequently observed minute foci of fat necrosis appearing about five hours after the process had been started, involving at first but one or two cells, but later spreading so that they sometimes formed foci of a size readily visible to the naked eye. While in their earliest stages it was possible to determine that the starting-point was a cell in contact

on one side with a lymph space, which was readily seen, as all the lymphatic channels were greatly distended because of the inflammatory process. As there could be found in serial sections no other way for the necrotizing agent to pass from the surface to these areas, it seemed probable that the lymph had brought it from the eroded surface.

Vascular transportation is improbable because of the location of the secondary foci. One experiment only was made with intravascular injection, with entirely negative results. Five c.c. of water containing 0.25 gr. of pancreatin was injected into the femoral vein of a medium-sized dog. On the following day the dog appeared somewhat weak, but not in bad condition, and after twenty-four hours it was killed. Autopsy disclosed no lesions in the body, except inflammation at the point of injection.

The peculiar character of the lesions themselves, their formation in minute foci like miliary tubercles, is another characteristic of the process. This seems to be due entirely to local conditions. From the way the omentum and intestines lie in the abdominal cavity it is evident that any injected or extravasated fluid must lie in small nooks and crannies, and thus come in contact only with small areas of fat which alone suffer. In proof of this is the fact that when the amount of injected material is large and active, so that a large surface of omental fat comes in contact, the entire surface necroses *en masse* and bands of friable white fat are seen that bear no resemblance to the more common focal lesions except in color and consistence. In fat necrosis produced by placing wads of cotton saturated with pancreatin in contact with fat tissue, the necrosis occurs chiefly in lines and masses on the surface in contact. When fat necrosis arises from pancreatic lesions, the omentum and peripancreatic fat nearest the gland often form one necrotic mass, while farther away, where the fluid is present in smaller quantity and thus not affecting large surfaces, the focal character is seen. In experiments upon dogs, in which a feeble solution was used so that the resulting foci were few in number, they were usually almost entirely limited to the fold of fat that lies along the anterior surface of the peritoneum, where the injected fluid naturally gravitates in these quadrupeds.

The earlier writers found that in human fat necrosis the lesions tended to be limited by the normal septa of the fat, confining it to individual lobules, and they considered this lobular character of some importance. It was found in these experiments that when spreading fat necrosis reached connective tissue septa it stopped there, although the connective tissue was often somewhat involved in the necrosis. However, the necrosis bears no relation to the septa further than this, and the septa only form the boundary when the necrosis spreads sufficiently to reach them; small foci are bounded by intact fat cells or by leucocytes.

#### SUMMARY

1. Fat necrosis seems to be merely a special form of necrosis of fat tissue, differing from simple necrosis chiefly in the sharp limitation of the affected area, usually by a wall of leucocytes and later by connective tissue; and the filling of the necrosed cells with products of fat-splitting. Each of these features can be produced experi-

mentally in various ways, but the complete picture has never yet been produced except by products of the pancreas.

2. Fat necrosis can be produced with constancy in cats, dogs, and rabbits by intraperitoneal injections of emulsion of fresh hog's pancreas.

3. Equally constant results can be obtained with ordinary commercial "pancreatins."

4. The results are the same with solutions in weak alkalies ( $\text{Na}_2\text{CO}_3$ ), weak acids ( $\text{CH}_3\text{COOH}$ ), or in water.

5. This property of the pancreatin to produce fat necrosis survives heating for five minutes at a temperature as high as some point between  $65^\circ$  and  $71^\circ \text{C}$ .; above this point the property is entirely lost. The amount of fat necrosis produced decreases steadily after exposures of  $55^\circ$  and upward. These observations point to enzyme action as the source of the condition.

6. Fat necrosis produced in this way is the same in appearance as human fat necrosis, both macro- and microscopically.

7. Dissemination outside the abdominal cavity has been observed as early as twelve hours after intraperitoneal injection. The route by which the spreading is accomplished is probably the lymphatic system.

8. The forms of the foci produced depend upon the areas exposed to the action of the pancreatin.

9. The earliest change in fat necrosis is a simple necrosis of the surface tissue, which extends gradually into the deeper fat cells.

10. Fat-splitting is subsequent to the necrosis, and not its cause. At first the products are non-crystalline, but become so later.

11. Calcification occurs probably by diffusion of the calcium salts from the fluids of adjacent intact areas.

12. The process progresses for but a few hours at any one point, the extension seeming to be limited by surrounding leucocytes.

13. Absorption of the area is accomplished by leucocytes, and healing is by proliferation of connective tissue from the margins. Adhesions are seldom formed.

14. The foci become visible to the naked eye in three to five hours. They may disappear within eleven days, or persist for a much longer time, depending chiefly upon their size.

15. Fat necrosis by itself is not dangerous to the affected animal, and may cause no observable symptoms.

16. The lipase of the fat tissue does not destroy the fat when the tissue has been made necrotic and preserved from outside influences of absorption, etc.

17. Simple alkaline solutions of the strength of pancreatic juice, or slightly stronger [ $\text{NaOH}$ ,  $\text{Ca}(\text{OH})_2$ ,  $\text{Na}_2\text{CO}_3$ ], do not produce fat necrosis.

18. Many of the features of fat necrosis may be produced after death in animals, and also *in vitro*, with pancreatin, but the resulting condition does not resemble fat necrosis at all closely.



## **OOGENESIS IN SAPROLEGNIA**



## OÖGENESIS IN SAPROLEGNIA

BRADLEY MOORE DAVIS

ALTHOUGH *Saprolegnia* is a form of considerable interest in connection with the problem of the so-called multinucleate gametes, nevertheless investigations have not been carried forward upon it with that attention to cytological detail that has recently been given to other Phycomycetes, *e. g.*, *Albugo*, *Peronospora*, *Pythium* and *Sclerospora*.

The present paper deals chiefly with the events of oögenesis and a comparison of this process with the development of zoospores. The material employed was apogamous, indeed apandrous, for specimens were chosen entirely free from antheridia to the end that the investigation might be relieved from the dispute on the sexuality of these fungi. However, as will be seen, the results have an important bearing on the well-known binucleate eggs, assumed by Trow to be stages of fertilization. At the end of the paper will be found an account, entitled "Theoretical Considerations," which deals with a number of topics suggested by this study in relation to recent investigations upon Phycomycetes and Ascomycetes.

The material was isolated in pure cultures and cultivated for several months on various substrata, during which time the writer had the opportunity of observing and confirming many of the adaptations recorded by Klebs (1899) in his detailed study of *Saprolegnia mixta*. In this period a number of structural peculiarities appeared, associated with the various sorts of nutrition, and forms arose presenting the characters of three closely related species, *Saprolegnia mixta*, *S. monoica*, and *S. ferax*. The variation was most marked in respect to the presence, absence, or relative quantity of antheridia which are the most important distinguishing marks of these species.

The original collection bore oogonia with relatively few antheridia (*Saprolegnia mixta*), and frequently none. By cultivating the form on a rich substratum—raw beef or fresh insects—a much more extensive growth of antheridial filaments was obtained, as in *Saprolegnia monoica*. On other media—boiled whites and yolks of eggs and dried beef—the filaments never produced antheridia, but oogonia were formed abundantly (as in *Saprolegnia ferax*), normal in size and with numerous oospores. After three months all cultures ceased to develop antheridia and the number of oogonia steadily decreased until the cultures reproduced entirely by zoospores.

But it was always possible to get oospores, as Klebs (1899) has shown, by placing cultures developing zoosporangia under such conditions that the hyphæ were no longer submerged. This may readily be done by removing material from water and placing it in a dish of cold agar-agar which will furnish enough moisture to support the fungus for several weeks. The filaments out of water promptly developed oogonia,



even when they had the form characteristic of zoosporangia. Such cultures frequently showed club-shaped oogonia whose eggs were arranged approximately in a line.

Chromacetic acid proved to be the most satisfactory fixing agent, but it must be employed much weaker than the usual formula. One per cent. chromacetic acid caused immediate contraction of the protoplasm, but a solution one-fourth per cent. chromic and one-tenth per cent. acetic acid gave excellent results, and presented advantages of clearness and preservation over weak Fleming, Merkel, corrosive sublimate, sublimate acetic, iridium chloride, or picric acid. Paraffin sections were cut 3-5  $\mu$  thick, and generally stained with safranin and gentian violet. The protoplasmic structures are so minute as to require lenses of the clearest definition, and the Zeiss apochromatic objectives 2 mm. and 1.5 mm. with the compensating oculars were employed throughout the investigation.

#### OOGENESIS

The accounts of nuclear and cytoplasmic activities in Saprolegnia during oogenesis present some striking contradictions, and leave untouched some phases of a detailed but very significant character. Humphrey (1892) was the first author to apply methods of cytological technique, cutting sections in paraffin, and his studies were followed by the investigations of Trow (1895, 1899) and Hartog (1895, 1896, 1899). The last two authors have expressed very divergent views, asserted with a positiveness that invests their discussions with an atmosphere of personal criticism that need not be reviewed in this paper. It is necessary, however, to consider certain conclusions of the earlier authors with which the present writer cannot accord, and it seems best to do this at the outset, leaving the points of agreement with the present investigation to be taken up in their proper connections.

It is well known that the oogonium of the Saprolegniales contains many times more nuclei than the number of eggs ultimately formed. Humphrey and Hartog believed that the nuclei fuse with one another, thus reducing the sum total until the requisite number was present. Trow stated that the number was diminished through degeneration and digestion until it was so small that each egg took but a single nucleus. The writer has found no evidence of nuclear fusions as reported by Humphrey and Hartog, and in general supports Trow's view of degeneration. However, there seems to be a reason, not known to Trow, for the selection of the fortunate nuclei destined to preside over the eggs, and a large part of this paper will deal with that matter.

It is also well known that the eggs of the Saprolegniales are not infrequently binucleate and sometimes trinucleate. Humphrey and Hartog considered such conditions as merely the final stages in that process of general nuclear fusion, the last pairings whereby the eggs become uninucleate. Trow has made much of these binucleate eggs, believing the two nuclei to be sexual and one of them introduced by an antheridial filament. He has been bold enough to assert sexuality for four members of the

group: *Saprolegnia declina*, *S. mixta*, *Achlya Americana*, and *A. Americana* var. *cambrica*. Nevertheless, Trow presents very little evidence that the so-called "male" nucleus comes from the antheridial tube, or that the latter structure ever opens into the eggs. The writer cannot justify Trow's conclusions in this matter, believing them premature as to evidence and illogical as to probabilities. The present study will attempt to show that binucleate and trinucleate eggs are to be expected under the peculiar conditions governing oogenesis.

With respect to cytological details, investigations scattered over so long a period as twelve years could hardly be expected to agree. Hartog studied from entire mounts, yet was able to count chromosomes and observe nuclear figures. Trow sectioned in paraffin, and was at first (1895) completely deceived as to the interior structure of the nucleus and the number of chromosomes. In his second paper, however, Trow (1899) concedes that the nuclei in the antheridia and oogonia divide mitotically, but his figures are far from clear as to detail. Trow was also mistaken in his interpretation of the nucleolus.

The present study will give a more detailed account of nuclear structure and activities than any previous paper. But the most important contribution relates to certain cytoplasmic manifestations that seem to determine in large part the results of oogenesis. These cytoplasmic activities place the process of oogenesis in *Saprolegnia* in new light, bringing it into sympathy with conditions in *Albugo*, *Peronospora*, and *Sclerospora*. They are concerned with that cytoplasmic structure termed the *coenocentrum*.

It is not strange that Humphrey, Hartog, and Trow failed to find the *coenocentrum*, for its recognition demands exceptionally good fixation and staining. It is probable that Dangeard saw it when he described an oil globule or fatty mass in the center of the egg. It seems possible that Trow may have mistaken it at times for a centrally placed nucleus, to which it bears a certain resemblance that might make the two structures indistinguishable in obscurely stained preparations. The *coenocentrum* does not appear until the processes of oogenesis are well under way. Previous to this period there are nuclear and cytoplasmic activities of considerable import, and they will be considered first.

It is well known that with the flow of the protoplasm into the swollen tip of a hypha there is apparent that peculiar structure of the protoplasm (Plate XV, Fig. 1) significant of its streaming movement. The nuclei at that time are very small. When the oogonium is cut off by a septum from the hypha that bears it, the protoplasm becomes distributed almost homogeneously through the interior (Fig. 2). The nuclei then increase in size and shortly after show most clearly that detail of structure that is to be expected in the resting nucleus. This structure agrees with the accounts of Harper, Wager, Stevens, and myself for the nuclei in other types of fungi, indicating that the conditions among these lower forms are essentially similar to the nuclear structure of higher plants. As is shown in Figs. 3 and 4 and especially in

Fig. 6 (Plate XV), there is a nuclear membrane inclosing a well-differentiated nucleolus, prominent by its size and staining qualities. Much less conspicuous, but readily demonstrated in well-fixed material, is a loose linin network which contains the chromatic material. Trow's description of a central body containing chromatin and nucleolar matter, but "neither a nucleolus nor a chromosome," must have been founded on inferior preparations. There are certainly no complexities in *Saprolegnia* comparable to the so-called nucleolus of *Spirogyra* (Mitzkewitsch, 1898, or Wisselingh, 1900).

There is one mitosis in the oogonium, but previous to that event a number of vacuoles are developed which generally result in a peripheral arrangement of the protoplasma around a large central space or vacuole containing cell sap. The vacuoles begin to appear immediately after the oogonium is cut off from the parent hypha (Fig. 2). They grow larger and run together as bubbles do in soapsuds (Fig. 3), until finally there are one or perhaps two large vacuoles in the center, and occasionally smaller ones near the edge (Plate XV, Figs. 10 and 11). The protoplasm then lies as a thick peripheral zone, and the nuclei (Fig. 5) are distributed around at varying distances between the oogonial wall and the boundary of the vacuole.

This is the period when one may expect to find the nuclei in mitosis. The event happens to most nuclei at about the same time, and good preparations of this stage of oogenesis are very striking (Fig. 5). The oogonium will be filled with the diamond-shaped spindles inclosed in nuclear membranes. Three stages of mitosis are shown in Figs. 7, 8, and 9. It will be noted that the spindle is intranuclear. Fig. 7 presents the condition just previous to metaphase, with the chromosomes, four in number, at the nuclear plate and the nucleolus lying outside of the spindle. Fig. 8 is of a stage shortly after metaphase, when the two sets of daughter-chromosomes have separated and are about to pass to the poles; the nucleolus is still present, but smaller and staining faintly. Fig. 9 is of anaphase, the two groups of daughter-chromosomes, four in each, lying at the poles of the spindle and the nuclear membrane manifestly about to disappear. The nucleolus probably dissolves; at least I have never been able to follow it much beyond metaphase, but surviving, it would of course soon be lost in the granular cytoplasm after the breaking down of the nuclear membrane. Although granules are sometimes present at the poles of the spindles, the latter are generally entirely free from appearances that might suggest centrosomes.

It will be noted that this description of mitosis in *Saprolegnia* is similar in all essentials to the accounts of Wager (1896), Stevens (1899, 1901), and myself (1900) for *Albugo*; Wager (1900) for *Peronospora*; Miyake (1901) and Trow (1901) for *Pythium*; and Stevens (1902) for *Sclerospora*. These studies cover a wide range of forms and material. They agree in describing the spindle as always intranuclear and without centrosomes. The nucleolus is a structure always distinct from chromatic material and always, as far as we know, disappearing during mitosis by dissolution or extrusion into the cytoplasm. The chromosomes are derived from a linin network and

after mitosis the chromatin returns to the granular condition generally present in resting nuclei.

Following the mitosis, the oogonium passes into a condition that is exceedingly difficult to study. The number of nuclei has been doubled by the division, but the daughter-nuclei are much smaller than the parents. A comparison of Fig. 4 with Fig. 10 will illustrate well the change. It is not the small size, however, that makes the examination so difficult, but the fact that these nuclei very shortly show signs of degeneration. Almost all of the nuclei are affected. The nuclear membrane becomes indistinct, and its contents finally lie as granular matter in a clear area that resembles, and probably is, a vacuole. The granular matter is undoubtedly derived in large part from the nucleolus that fragments, but some of it may be chromatin. The study of the steps in this process of general degeneration is especially baffling because the progress is toward a time when the nuclear material becomes indistinguishable from other granules in the cytoplasm.

It is difficult to understand how Humphrey and Hartog could ever have interpreted this process of degeneration as successive nuclear fusions. As Trow pointed out, successive fusions should give more and more conspicuous nuclei, as the material accumulated with each union, and consequently an ever-increasing clearness of conditions. In reality, however, we pass from the stage illustrated by Fig. 10 just after mitosis, to the vague conditions presented in Figs. 11, 14, and 15 (Plate XV). The last two figures are of oogonia much older than those shown in Figs. 10 and 11, and illustrate late stages in the process, when the nuclear membranes have mostly disappeared and the nucleoli and possibly chromatic material lie in vacuoles. Such vacuoles are frequently elongated, and when they contain two masses of deeply staining material there is suggested a stage in nuclear fusion, and such appearances probably deceived Humphrey and Hartog. However, the vagueness of structure and manifest waning of the previous clear definition should have put these observers on their guard. These degenerate nuclei remain for a long time, even after the eggs are fully formed, and it is quite impossible to tell with exactness when they lose their structure and functions.

The eggs are formed during the process of nuclear degeneration described above, and their nuclear structure is really determined by that event. Trow (1899) has given us a very good account of the general stages in this process of protoplasmic segmentation, but he did not know the cytological details, and there is reason to believe that he may have been mistaken in his interpretation of certain structures which he considered nuclei. The first external indication of protoplasmic segmentation is the gathering of the contents of the oogonium into denser masses around certain centers, these masses projecting into the central vacuole and destroying that even outline present in earlier conditions of the oogonium (Fig. 5). The protoplasm between the egg origins is less dense, and presently begins to develop small vacuoles (Fig. 12) which run together until the egg origins are separated by spaces of considerable size (Fig. 13). Many of these vacuoles break through the films of protoplasm into the central space, which

then appears to have put out extensions toward the cell wall. The protoplasm of the oogonium is exceptionally mobile at this time, and the vacuoles are constantly changing their forms and positions. In the end the protoplasm gathers more and more closely around the centers of the spore origins, and finally the latter break away from one another at all points of mutual contact (Fig. 13), and the several independent protoplasmic masses round themselves off as eggs.

The reader will have noticed in the illustrations of this protoplasmic segmentation that each egg origin has a deeply stained center surrounded by delicate rays (Figs. 12-15). These star-like structures are very conspicuous under low magnification (in Figs. 12 and 13, 500 diameters), when the center appears to be a single structure. In reality it is not a simple unit, but is always composed of at least two structures, a cœnocentrum accompanied by a nucleus. This dual nature is made clear only under high magnification, with clear preparations of very thin sections. I do not think it would be possible to understand the structure from entire mounts such as Hartog's. Hartog probably considered the center as a nucleus alone, and certain of Trow's figures indicate that he gave a similar interpretation. The cœnocentrum is really the key to many of the problems of oogenesis in Saprolegnia.

The cœnocentrum varies in its minute structure with different periods of oogenesis. It is at first a small body composed of several granules imbedded in dense material, from which a number of delicate fibrils radiate into the surrounding cytoplasm. The structure stains deeply and resembles an aster. After the eggs are fully formed the rays disappear and the cœnocentrum grows larger, takes on a spherical form, and resembles a globule of oil or fat. The cœnocentrum finally dissolves, sometimes with fragmentation, and completely disappears in the older eggs. The cœnocentrum is then a structure peculiar to that period of oogenesis characterized by nuclear degeneration and the segmentation of the protoplasm to form the eggs. It bears a most important relation to these two events, which are the most difficult to study in the entire process of oogenesis.

We must begin with the first appearance of the cœnocentra. These structures may always be found before the differentiation of the egg origins, at the time when the oogonium is filled with degenerating nuclei. The latter lie scattered through the cytoplasm, as is shown in Figs. 14 and 15, and exhibit varying degrees of dissolution. The young cœnocentra are always found in the densest regions of the protoplasm, portions destined to become egg origins, such as are shown in Figs. 14 and 15. They are very small at first and would scarcely be noticed except for the radiating fibrils that mark their position. They increase in size as the egg origins take more definite form (Fig. 16, Plate XVI).

An examination of Figs. 14, 15, and 16 will show at the side of each cœnocentrum a small nucleus. This structure is very small at early periods of oogenesis (Figs. 14 and 15) and scarcely more clear than many of the degenerating nuclei in the neighborhood. But as oogenesis proceeds the nucleus accompanying the cœnocentrum

grows larger and increases greatly in staining material (Fig. 16). When the eggs are fully formed this nucleus is many times larger than at the first appearance of the cœnocentrum, as may be seen by comparing Figs. 17-21 (Plate XVI) with Figs. 14 and 15, which are all magnified 1,000 diameters. One would hardly think it possible that the large nucleus present in the center of the mature egg was ever so small as the degenerating nuclei whose remains may be found in advanced stages of oogenesis (Fig. 16), and sometimes even in the fully formed eggs (Figs. 17 and 23). But there seems to be no doubt of this. The nucleus destined to preside over the egg is at first indistinguishable in size or structure from many of its neighbors.

What should lead to its selection as the egg nucleus? I can see no other explanation but that its position gives it dynamic advantages, enabling it to survive when its neighbors lack the metabolic conditions necessary for nuclei and consequently must degenerate. This conceives the oogonium as too richly stocked with nuclei for the metabolic conditions of oogenesis, and in consequence the field of a struggle of the parts ("der Kampf der Theile," Roux).

What is the relation of the cœnocentrum to these events? As we have stated, the cœnocentrum is not a permanent organ either in the oogonium or the egg. It appears with the first indications of the egg origins and passes away as the eggs grow older. It is obviously a transitory structure peculiar to the most active periods of oogenesis. To the writer the cœnocentrum seems to be the morphological expression of dynamic activities in the oogonium, and especially in the egg origins at the time when these are differentiated. The cœnocentrum has the appearance of being the focal point in the center of the egg origins of the metabolic conditions peculiar to oogenesis. And this offers a very plausible explanation of the survival of the nucleus which lies nearest the cœnocentrum.

The nucleus most fortunate in its position near the cœnocentrum should be greatly benefited if this is a region of the protoplasm more favorably nourished than other parts. It is probable that the cœnocentrum even draws toward itself nuclei within a certain sphere of attraction. Nuclei may be found with a pointed end extended toward the cœnocentrum (Figs. 16 and 20, Plate XVI). It will be remembered that Stevens (1901, showed with great clearness for *Albugo candida* and *A. Tragopogonis* that the nuclei in the immature eggs stretch toward the cœnocentra so that their long dimensions are frequently twice the width. The nuclei of *Saprolegnia* are too small to present conspicuous morphological evidence of this character. But we have the fact that the favored nucleus is almost always pressed against the cœnocentrum which, together with the appearance of the nuclei and what we know of the events in *Albugo*, makes it quite certain that the cœnocentrum exerts a chemotactic influence.

The changes that come over the egg as it matures are illustrated in Figs. 16 to 21 (Plate XVI), which show the usual uninucleate condition of the egg. Binucleate and trinucleate eggs will be described in the following paragraphs. The two most important events of maturation are the increase in size of the nucleus and the gradual dissolution

and final disappearance of the cœnocentrum. The growth of the nucleus involves not only the extent of the space inclosed in the nuclear membrane (Figs. 17-21), but also means a great increase in the amount of staining material, chromatic and nucleolar. The latter must be very many times greater in quantity in old eggs than at the beginning of oogenesis (compare Fig. 16 with Figs. 20 and 21). The cœnocentrum decreases in size until it becomes a very small globule (Fig. 20), or it may split up into several granules, which soon become lost in an ill-defined mass of denser protoplasm. The cœnocentrum finally disappears, and the contents of the egg then arrange themselves around a central vacuole, with the nucleus taking a peripheral position. This is the structure of the mature egg, and is illustrated in Fig. 21.

We will now consider some conditions that have given rise to much discussion, namely, the binucleate and trinucleate eggs. They have been found by Humphrey, Hartog, and Trow, and the present study indicates that they may be expected in any member of the Saprolegniales. Trow attached much significance to them as evidence of sexuality, but his conclusions seem to the writer open to much criticism and will be taken up presently. Figs. 22-5 (Plate XVI) illustrate several conditions that show how easily an egg may become binucleate. Suppose two nuclei lie near enough to the cœnocentrum to share about equally the advantages of position. Then it is not likely that either will give way to the other. Such conditions in a young egg are shown in Fig. 22. Fig. 25 represents also a pair of nuclei one above the other and both extended toward the cœnocentrum, which was fast breaking down. Fig. 24 is very interesting. In this instance the cœnocentrum is the center of a mass of protoplasm considerably larger than the average egg. There are two well-developed nuclei, and the form of the cell suggests the probability that material which ordinarily would have gone into two egg-origins has been held together in this instance by the influence of an especially large cœnocentrum. An illustration of quite the reverse condition is shown in Fig. 23, and is remarkable. Here we have presented an egg with two cœnocentra, and at the side of each a nucleus. There is no doubt from the age of the eggs that the two nuclei in each of these cases are sister nuclei. It is plain that the processes that work for the segmentation of the protoplasm in the oogonium are complex, not all in the influence of the cœnocentrum, nor yet all in the general activities of the cytoplasm.

Give the egg two nuclei with a fair start over their degenerating neighbors, and they seem to be able to exist side by side, not differing, as far as one may see, from the nuclei of uninucleate eggs. The two nuclei may lie far apart, as in Fig. 26, or so near together that they touch, as in Figs. 25 and 27. But in no instance—and I have seen a great many binucleate eggs—have I ever observed them fusing. Trow (1899) reported an instance of nuclear fusion in the egg, but the writer thinks we are justified in waiting for confirmations of this observation before attaching to it the importance given by that author.

Trinucleate eggs are somewhat rare in *Saprolegnia mixta*. I have seen hardly more than a dozen, and these were all rather mature examples. I have never been for-

fortunate enough to find young stages, periods comparable to Fig. 22, 23, or 24 of the binucleate eggs. The three nuclei may be grouped close together in the egg (Fig. 28), or may lie quite separate from one another (Fig. 29). There is no evidence that they fuse. The rather meager data at hand indicate that when there are three nuclei in an egg they are individually smaller than the single nucleus in an ordinary egg (compare Figs. 28 and 29 with Figs. 19-21). This is to be expected, for in general the three nuclei share between them the metabolic possibilities of about the same amount of protoplasm as is in the uninucleate egg. The trinucleate egg probably develops, as does the binucleate, from an egg origin in which more than one nucleus by fortunate position is able to survive the processes of general degeneration.

Let us now examine Trow's position respecting sexuality in the Saprolegniales. They are presented most completely in his 1899 paper. I approach this subject with some diffidence, for it has already been the occasion of detailed discussions of a personal character (Hartog, 1896, 1899). The matter finally boils down to a question of confidence in Trow's evidence, his account, and his figures. Everyone must admit the possibility of sexuality in the Saprolegniales, but the question for us is: Does Trow prove it?

The binucleate egg gave Trow the conviction, as he acknowledges, that fertilization took place through the introduction of a male nucleus into the egg from an antheridial tube. But the present studies show that binucleate eggs are quite common in an undoubted apogamous form, the material being entirely free from antheridial filaments. Moreover, these binucleate eggs have been followed through younger stages back almost to the period of the egg origins, and we know that these two nuclei were sisters in the oogonium. To make this point more plain, let the reader contrast the appearance of the two small nuclei shown in Figs. 22 and 23 with the nuclei in older eggs (Figs. 19, 20, and 21) and it will be evident that the former have the size and structure of nuclei in the young oogonium, and not of the fully mature gamete (egg) nucleus. It should also be noted that Hartog's binucleate eggs were from apogamous material (Hartog, 1898 and 1899, p. 450) as were also mine.

If, then, apogamous material may have binucleate eggs, and the events of oogenesis explain the conditions, we are justified in examining Trow's evidence of sexuality very critically and demanding of it exceptional fulness and accuracy. We are concerned chiefly with Trow's figures, for they should show most exactly what the investigator really saw. I have been impressed with the lack of detail in many of these figures, which has led me to think that Trow may have made a number of mistakes which would quite invalidate his evidence in support of sexuality. Figs. 43, 44, and 46 (Plate XVI) give appearances labeled "female gameto-nuclei" which are very similar to coenocentra, and I fear that he was not able to separate these structures in his preparations. Fig. 35 certainly indicates that his material had coenocentra. But the most serious difficulties are encountered in his drawings of male gameto-nuclei (Figs. 45 and 46). These are not clear enough to be convincing; indeed, they seem to the writer to be the remains



of degenerating nuclei at the periphery of the egg. Side by side with the structures labeled "male gameto-nuclei" Trow figures bodies very similar in appearance, which are probably degenerate nuclei. In the face of this uncertainty and seeming contradiction of evidence the illustration of an antheridial filament piercing the egg (Trow, 1899, Fig. 45) loses much of its weight, and the statement that two nuclei fuse in the center of the egg (Trow, 1899, Fig. 47) is open to much doubt. The subject is so difficult that there are abundant opportunities for error, and we are justified in asking for much more evidence before accepting such important conclusions.

The writer cannot better sum up his attitude toward Trow's opinions on sexuality in the Saprolegniales than by defining them as *not proven* and *improbable* in the face of the mass of observations upon which botanists have generally agreed that the group is apogamous. The view of apogamy, formerly resting entirely on the failure to find antheridial tubes fusing with the eggs, is now supported by the present investigation on the details of oogenesis. These show that the binucleate egg, formerly difficult to understand on the theory of apogamy, may arise very naturally in a multinucleate oogonium when the method of oogenesis is as just described for *Saprolegnia mixta*.

The binucleate and trinucleate eggs of *Saprolegnia* are essentially similar to the multinucleate eggs of *Albugo Bliti* and *A. Portulacae*, and the conditions in the young eggs of *A. candida* and *A. Tragopogonis*, as described by Stevens (1899-1901). These latter, it will be remembered, contain several potential gamete nuclei, but, so far as we know, only one of these becomes functional. But it would not be surprising to find at any time binucleate or trinucleate eggs among species of *Albugo* that are normally uninucleate.

In concluding, we must lay emphasis on the importance of the cœnocentrum as an index of the activities peculiar to oogenesis in *Albugo*, *Peronospora*, *Sclerospora*, *Pythium*, and *Saprolegnia*. Although this structure is probably in large part the expression of activities of the protoplasm as a whole, still there can be no doubt of its material existence. It is difficult to understand how Trow (1901, p. 291) can question this point, except that his figures indicate that fine details of structure were not shown in his preparations.

It would be strange, indeed, if so large a mass of protoplasm as the cœnocentrum should not react in turn on the protoplasm that gave it birth. The cœnocentrum is not a mass of food material, even though much of its granular substance may be the products of metabolism, and the structure as a whole trophoplasmic in character. It is protoplasm, and as such must be counted a factor in the subtle processes of oogenesis. Trow's comparison of the cœnocentrum to a whirlpool in a river is not good, for there is unquestionably in this structure the expression of chemical phenomena as well as physical. The evidence is very strong from Stevens's (1901) work on *Albugo*, and the present study on *Saprolegnia*, that the cœnocentrum has a sphere of chemotactic influence on the nuclei in its neighborhood.

## SPOROGENESIS

Except for a recent paper by Timberlake (1902) on *Hydrodictyon*, we know little of the details of zoospore formation in either algæ or fungi, and the field would certainly repay investigation. The writer examined the sporangium of *Saprolegnia mixta* to contrast the conditions there with the processes of oogenesis, but little came of the study, the subject not being favorable, except a general confirmation of the accounts of sporogenesis given by Rothert (1888), Hartog (1888), and Humphrey (1892). If the oogonium is the homologue of the sporangium, we should expect a general similarity in the protoplasmic activities of each structure. There is the general agreement that the protoplasm segments by cleavage planes determined chiefly by vacuoles. But beyond this the activities of the two structures have little in common and a great many peculiarities.

As is well known, there is no mitosis in the sporangium. A large number of nuclei are carried into the tip of the hypha by the accumulation of protoplasm there. Vacuoles collect and develop in the center of the young sporangium (Fig. 30, Plate XVI), and, flowing together, form a large central space inclosed in a vacuolar membrane (Fig. 31). The nuclei then lie scattered in the peripheral layer of protoplasm, and presently clefts appear which work outward between the nuclei from the central vacuole (Fig. 32). The clefts divide the protoplasm so that it is cut up into polygonal areas, with clearer regions between. These are the zoospore origins, and each contains a nucleus.

Rothert's explanations of succeeding conditions, which have also been confirmed by Humphrey and Hartog, seem entirely satisfactory. The sporangium is in a state of turgor when the clefts arise and push their way from the central vacuole toward the periphery. They finally reach the cell wall and immediately make possible the relief of the fluid in the central vacuole. There is at once a very evident decrease in turgor, which has an interesting effect on the appearance of the spore origins. The polygonal areas run together, and the whole sporangium becomes again almost homogeneous in structure. This means that the contraction of the sporangium brings the spore origins so close together that the clefts become almost obliterated. The spore origins also swell. They then begin slowly to separate preliminary to their final rounding off as zoospores. There is a period when the small masses of protoplasm form a very irregular network through the sporangium (Fig. 33), and this is followed by a more regular arrangement (Fig. 34), in which the spore origins are connected by very delicate protoplasmic strands. The latter are finally broken and the bodies round off as zoospores.

The writer searched persistently in the sporangium for cytoplasmic centers around which the process of segmentation might proceed, in the hope that light would be thrown upon the problem of the cœnocentrum, but the examination brought forth no evidence of such structures in the sporangium. The nuclei themselves seem to be the ultimate centers of segmentation. The cœnocentrum is then, so far as we know, a structure peculiar to the oogonium.

## THEORETICAL CONSIDERATIONS

The writer has once before (Davis, 1900) treated a number of topics suggested by recent studies on the Phycomycetes. The advances in this field of research, and also among the Ascomycetes, have been significant, and we seem to be nearing a point where much clearer conceptions of morphology and phylogeny may result. In this paper we will take up a number of considerations suggested by this and other investigations since 1900, and for convenience they will be grouped under headings as follows:

1. Homologies of the cœnogamete.
2. Origin and evolution of the cœnogamete.
3. Pyronema and cœnogametes among the Ascomycetes.
4. Phylogeny of Phycomycetes and Ascomycetes.
5. The nucleus of Phycomycetes in ontogeny.

## HOMOLOGIES OF THE CŒNOGAMETE

The writer suggested the term "cœnogamete" (Davis, 1900) as appropriate to fusing multinucleate masses of protoplasm whose individual nuclei are actually or potentially sexual. Stevens's first paper (1899) on *Albugo Bliti* really opened the field in its newer cytological aspects. Since then Harper (1900) has described for the Ascomycete *Pyronema* strikingly similar conditions, as has Juel (1902) for *Dipodascus*; and from the studies of Gruber (1901) we know more about the sexual processes in the Mucorales. Harper's results will be considered in a special connection. It is important at the outset that we understand clearly the homologies of the cœnogamete.

Are all cœnogametes homologous with one another, and from what have they been derived among the algæ? It will be agreed that the Mucorales, *Albugo Bliti*, and *Pyronema* illustrate completely the conception of a cœnogamete. It is part of our problem to determine the relation of these conditions to the sexual organs in other species of *Albugo*, and in *Peronospora*, *Pythium*, and the Saprolegniales. There may be some hesitancy in following the series of homologies that the writer shall propose, and the evolutionary history to be suggested, but he can see only two possibilities, and one of these so obscure that it seems almost impossible in the light of our present knowledge, incomplete as it is.

The most important structures in the cœnogamete are the nuclei, and there can hardly be any question but that they individually stand for energids that among the algæ are independent uninucleate gametes. Stevens's (1899) term "compound oosphere" expressed very well this conception of the conditions in *Albugo Bliti*. It was employed when this form was the only type known presenting the structure implied by the phrase, and these conditions might have been purely exceptional. But we now know from later studies of Stevens (1901) that other species of *Albugo* (*A. Portulacæ*, *A. Tragopogonis*, and *A. candida*) have phases of ontogeny identical with the essential periods of oogenesis in *A. Bliti*, and may be brought into very intimate relation to the latter species. We also know that the cœnogamete is not

restricted to the Peronosporales, but is characteristic of the Mucorales, and is found also among the Ascomycetes. It is not likely that we shall retain the phrase "compound oosphere," for a broader conception will probably take its place, but a purpose has been served and a field opened to investigation that was quite undreamed of by the earlier investigators of the Phycomycetes.

The nuclei of cœnogametes are homologous with nuclei in a gametangium destined to develop independent sexual cells. Hartog's (1891) conception of the nuclei in the periplasm of Peronospora as representing degenerate gametes has been completely justified, and there are very good reasons for believing that the nuclear divisions in the oogonium and antheridium of the Saprolegniales and Peronosporales are "phylogenetic reminiscences of the formation of gametes." The attempts to establish special functions for these mitoses as reduction divisions for the eggs have been inconclusive.

The oogonia and the antheridia of the Peronosporales, Saprolegniales, and Pyronema are the homologues of gametangia, and consequently of that simplest type of cœnogamete, as illustrated in the Mucorales. There is everything in the morphology of these structures to favor these conclusions, but only recently have we known the details of protoplasmic organization. When an entire gametangium functions as a gamete, as in the Mucorales, it becomes a cœnogamete. In Pyronema, Albugo, and the multinucleate eggs of the Saprolegniales the cœnogametes are restricted portions of the protoplasm in such gametangia, but it is obvious that in Pyronema and Albugo the gametangium behaves as a whole in a manner strictly similar to the fusion of the cœnogametes in the Mucorales. It should be noted that these homologies are quite independent of the problem of the origin of the cœnogametes in the various groups. That topic will be treated in the next section of the paper.

Stevens (1901) has carried the homologies a step farther in suggesting that the receptive papilla from the oogonium of the Peronosporales marks the position of the pore that develops in the gametangia of algæ to give entrance or exit to the sexual elements. This is a very interesting comparison and is worth following to its limits. Thus the points of fusion of the cœnogametes of a mould may be homologous with the points of exit of the motile gametes from the gametangium of some algal ancestor.

The term "cœnogamete" should be employed in the strict sense indicated when the term was proposed (Davis, 1900, p. 307). It is a structure containing more than one gamete nucleus, and generally very many functional or potential gamete nuclei. It is generally homologous with a gametangium, the binucleate and trinucleate eggs of the Saprolegniales, and the multinucleate eggs of *Sphaeroplea annulina* var. *Braunii* (Klebahn, 1899) presenting the only exceptions, for the oogonia of *Albugo Bliti*, *A. Portulacae*, and *A. Tragopogonis* really acts as a whole, and it is hardly possible to separate in these forms the cœnogametes (oospheres) from the gametangia. When we say that the oogonium of Albugo, Peronospora, Sclerospora, and Pythium acts as a whole we mean that the periplasm is not to be considered as waste material, but as a specialized region of the cell, with important functions in relation to the eggs, which

it helps to protect by assisting in the formation of heavy walls. The Mucorales, Pyronema, and these three species of Albugo furnish the best known illustrations of cœnogametes.

#### ORIGIN AND EVOLUTION OF THE CŒNOGAMETE

There seem to be only two possible sources of the cœnogamete. It is conceivable that a uninucleate sexual element might become multinucleate, perhaps through such an increase in the protoplasmic content that more than one nucleus would be required to control satisfactorily its activities. The second possibility is an origin from a multinucleate gametangium that has given up the production of uninucleate gametes, and acting as a unit becomes itself a sexual organ, a cœnogamete. Such an evolutionary process would find its analogy in those sporangia (conidia) of certain species of Pythium and Peronospora, which now germinate as a whole (by a tube) instead of forming zoospores.

The first possibility has absolutely no evidence in its support. There is no series of forms whose sexual cells pass from a uninucleate condition to a multinucleate. There are no indications that such an evolutionary process has ever taken place among plants. There are only two instances known where eggs, free from periplasm, are multinucleate. The eggs of Albugo are so intimately associated with periplasm that they cannot be considered apart from the gametangium in which they lie. These two examples are the binucleate and trinucleate eggs of the Saprolegniales and the multinucleate eggs of *Sphaeroplea annulina* var. *Braunii*. Our investigations of Saprolegnia have shown that the processes of oogenesis in that group have as an end the sacrifice rather than the preservation of nuclei, and the uninucleate condition is evidently the goal of evolution. Klebahn's (1899) and Golenkin's (1899) studies of *Sphaeroplea* are incomplete in certain cytological details of oogenesis, and the fact that the eggs of some forms are uninucleate suggests caution before laying emphasis on the multinucleate condition. It is possible that further study will relate the multinucleate eggs to the uninucleate, as in Saprolegnia.

What evidence have we of the second possibility, *i. e.*, the origin of the cœnogamete from a multinucleate gametangium which, ceasing to form uninucleate sexual cells, becomes itself a cœnocyctic gamete? Most important is the exceedingly interesting series of four species of Albugo described with so much detail by Stevens (1901). We cannot take up this investigation except to notice that the four species form a well-graded series in which the evolutionary direction is clear and very important for the conclusions that we are striving to establish. The oospheres of *Albugo Bliti* and *A. Portulacae* contain many functional gamete nuclei, that of *A. Tragopogonis* several potential and several functional, and that of *A. candida* several potential and one functional. In this series the cœnocentrum is very small in *A. Bliti* and *A. Portulacae*, larger in *A. Tragopogonis*, and very large and strongly chemotactic in *A. candida*. A fifth form has been added to this series by Ruhland (1902), who finds that *Albugo Lepigoni* is even more highly specialized than *Albugo candida*, since it contains an

extraordinarily large cœnocentrum. The evolution in complexity is plainly from *A. Bliti* to *A. candida* and *A. Lepigoni*, that is, from the multinucleate egg to the uninucleate. And this series offers the most striking evidence against the evolutionary possibility considered in the previous paragraph.

Now, the multinucleate eggs of *Albugo* are not the most primitive types of cœnogametes, because they contain only a portion of the total number of nuclei in the gametangium, many of the sister-nuclei passing into the periplasm. They are not as simple as the cœnogametes of the Mucorales, nor yet as primitive as the oogonium of *Pyronema*, which has no periplasm, although it sacrifices a large number of nuclei in the conjugating tube (trichogyne), and by this specialization presents conditions more complex than the molds.

It is the specialization of a periplasm simultaneous with the reduction in the number of functional gamete nuclei that has made possible the elaborately organized oogonium of *Peronospora*, *Sclerospora*, *Albugo*, *Araiospora*, and to a lesser degree *Pythium*. And the cœnocentrum is perhaps most largely responsible for the highest degree of specialization. The cœnocentrum largely influences and perhaps controls the position and structure of the eggs. The larger the cœnocentrum, the more direct is the effect on neighboring nuclei, and the greater is the benefit to such nuclei as are fortunate to be within its sphere of operations. So in the struggle for existence among potential gamete nuclei in the oogonium, the cœnocentrum has a power of assistance that, according to its degree of development, determines the structure of the egg, whether multinucleate or uninucleate. The evolutionary trend is physiologically precisely the same as is shown among the algæ (*Fucales*, *Vaucheria*), when potential gamete nuclei are sacrificed to provide functional nuclei with a large amount of richly nourished protoplasm.

But it should be noted that, although the evolutionary processes in the *Peronosporales* have resulted in uninucleate eggs, these structures are not strictly homologous with the eggs of algæ. They are homologous only in the sense that the eggs of *Volvox*, *Fucus*, *Vaucheria*, *Chara*, and several other highly developed algæ are homologous. In these algæ the eggs have an ancestry from much simpler types of gametes, and relationships must be traced through these or perhaps through older forms of asexual spores. The oogonium of the higher *Peronosporales* has come through a series of cœnogametes of which *Albugo Bliti* represents a certain stage, but whose earlier forms must have been simpler. The primitive conditions probably had a structure comparable to the cœnogametes of the Mucorales, and that type of structure finds its nearest approach among the algæ in the gametangia that discharge numerous gametes, as illustrated by *Cladophora* and many of the *Siphonales*.

But it will immediately be asked: What are we to do with such algal types as *Vaucheria*, *Sphæroplea*, *Cedogonium*, etc.? Have they no relation to the fungi? This will be considered under the topic "Phylogeny of the Phycomycetes and Ascomycetes." It is important that we emphasize now the evolutionary process brought out by

Stevens's work on the four species of *Albugo*, and extend the results of that study to the Peronosporales as a whole. Accordingly, we have good reason to believe that the uninucleate eggs of *Albugo candida*, *Peronospora*, and *Pythium* have not been derived from the eggs of algal ancestry, but from cœnogametes which passed through the stage illustrated by *Albugo Bliti*, and came from much simpler conditions, probably resembling in many respects the cœnogametes of the moulds and *Pyronema*.

An origin of the simplest types of cœnogametes (moulds and *Pyronema*) from gametangia of algæ presents certain difficulties that should be discussed. The process would involve a change in the activities of a structure from one where the nuclei show a considerable degree of independence to one in which the nuclei co-operate in a cœnocyctic cell that acts as a unit. An evolutionary process comparable to the above must have taken place with the development of the multinucleate zoospore of *Vaucheria* if its nuclei stand for the numerous zoospores generally formed in the terminal sporangia of the Siphonales. And a similar evolution, as has been mentioned before, is shown in the development among the Peronosporales of conidia (which germinate by tubes) from sporangia (conidia) that form zoospores. Such conidia and the zoospores of *Vaucheria* are not considered the equivalent of tissues, but units in their physiological behavior, just like uninucleate spores.

Similarly, the cœnogamete is not the equivalent of a tissue, and must not be considered as made up of independent gametes associated together because their cytoplasm is fused into a common mass. It exhibits the same sort of individuality as any cœnocyctic cell or structure. We no longer draw sharp lines between uninucleate and multinucleate cells, for we realize that the transformation of the first into the second is a very simple matter, and that the unity of the cœnocyte is not disturbed by its having several or many nuclei, for these do not occupy fixed positions in the cell, but wander with the varying movements of the protoplasm. The cœnogamete is as much an individual cell as the uninucleate gamete, and distinctions can no more be drawn between these two structures than between the adjacent uninucleate and multinucleate cells of many plants (*Chara*, *Rhodophyceæ*, etc.). In view of its structure and behavior the term "cœnogamete" seems to the writer appropriate.

#### PYRONEMA AND CœNOGAMETES AMONG THE ASCOMYCETES

Harper's (1900) investigation of *Pyronema* has established a condition in the Ascomycetes very similar to that among the Phycomycetes. *Pyronema* has as conspicuous a cœnogamete as the Mucorales or *Albugo Bliti*. Its peculiarities do not affect the essential cytological structure of the fusing multinucleate masses of protoplasm whose gamete nuclei unite in pairs as in *Albugo Bliti* and probably in the moulds. The sexual apparatus of *Pyronema* differs from the moulds chiefly in the development of that specialized structure the conjugating tube (trichogyne). This organ is manifestly of advantage because it affects a union with the antheridium and probably, as Harper suggests, represents the same sort of outgrowth from a sexual element as a

trichogyne. The nuclei in the conjugating tube break down, and the structure finally becomes merely the channel through which the protoplasm from the antheridium flows into the oogonium. This movement of the protoplasm is very similar to *Albugo Bliti*, and the resemblance is carried still farther in the distribution and fusion of the gamete nuclei in pairs throughout the oogonium. The "receptive papilla" of *Albugo* is developed from the oogonium, and the conjugating tube in *Pyronema* may be considered an elaboration of such a growth tendency. Periplasm is lacking in *Pyronema*, but the mass of nucleate protoplasm that passes into the conjugating tube may relieve the oogonium of those conditions that result in the extensive degeneration of potential gamete nuclei in *Saprolegnia* or the somewhat similar conditions affected by the differentiation of a periplasm in the Peronosporales.

It is not to be supposed that the cœnogametes of *Pyronema* are closely related to those of the Mucorales or the Peronosporales, excepting as all of these structures are the homologues of gametangia. But it is important that we should recognize this condition among the Ascomycetes as one that further study may show to be not uncommon in the group. Juel (1902) reports it for *Dipodascus*. Miss Nichols's (1896) studies on *Ceratostoma*, while inconclusive in cytological details, are of importance in this connection. She has described and figured multinucleate oogonia (archicarps) and antheridia, which are said to fuse. They are apparently cœnogametes, and it is probable that these structures will be found in other genera of the Pyrenomycetes and Discomycetes. There are several forms whose archicarps suggest a cœnocyctic structure (*Eremascus*, *Ascobolus*, *Sordaria*, *Erotium*, etc.).

The student of the homologies and evolution of the sexual organs among the Ascomycetes now finds himself face to face with the same problem that has been presented to Stevens and myself for the Phycomycetes. What is the relation of the uninucleate gamete (*e. g.*, *Sphærotheca*) to the multinucleate? Which condition is the more primitive?

There is likely to be some confusion of homologies among the sexual organs of the Ascomycetes. The oogonium (archicarp) of *Sphærotheca* is morphologically a gametangium, and so is the antheridium, but both structures are physiologically gametes. The oogonium and antheridium of *Pyronema* are morphologically gametangia, so that in comparing these two forms we are dealing with homologous structures. Of course, we use them merely as illustrating certain sexual conditions; indeed, they are almost the only Ascomycetes whose sexual organs have been thoroughly studied, with the exception of certain lichens and the Laboulbeniaceæ, where the conditions are very different and which will be considered later.

The problem then will be: Did the uninucleate condition of the gametangium, as represented by *Sphærotheca*, come from a multinucleate gametangium (cœnogamete) illustrated by *Pyronema*, or is it the progenitor of the latter? We have no series of forms in the Ascomycetes such as the four species of *Albugo* studied by Stevens to help us to a conclusion. But the problem in the Ascomycetes seems to be identical



with that of these Phycomycetes discussed in the previous sections of this paper. To derive a multinucleate gamete (Pyronema) from a uninucleate (Sphærotheca) involves an evolutionary process quite unknown to botany. To derive a uninucleate gamete from a cœnogamete merely demands a gradual reduction of the number of gamete nuclei, a process which we know to have taken place in several groups of algæ, independently of each other, and which is so beautifully shown in Stevens's series of four species of Albugo.

Harper (1900, pp. 388, 389) seems to be undecided as to the developmental relation of conditions in Sphærotheca to such as are presented in Pyronema. He shows that the oogonium of Sphærotheca could easily be given the form of Pyronema by the development of the beak into a conjugating tube with some minor changes in the position of the antheridium. But he disregards the internal changes necessary to derive a cœnogamete from a uninucleate gamete. And at the end of the same paragraph he says: "Still I am inclined to believe that the reverse process has taken place and that the sexual apparatus with the trichogyne represents the more primitive type for the Ascomycetes." To the writer resemblances of form have very little value in such comparisons, and relationships must be traced through agreement in the details of protoplasmic activities. And again, as Harper points out, the general morphology of the Erysiphæ is much higher than that of Pyronema. But Professor Harper by his last statement has warded off criticism, and perhaps, with the evidence from Albugo and Saprolegnia before him, he will feel more certain, with the writer, that the cœnogamete when related to the uninucleate gamete always represents more primitive conditions.

And this conception has a very interesting relation to the possibility of deriving the trichogynes of lichens and the Laboulbeniaceæ from a primitive type of sexual organ that may have been a cœnogamete. Of course, there is no reason why a uninucleate gamete (archicarp) among the fungi might not develop a simple trichogyne, as has been done in the Rhodophyceæ, but the trichogynes of the lichens and the Laboulbeniaceæ are generally systems of cells quite distinct from the female gamete (carpogenic cell). These conditions are nowhere presented in the red algæ, and it is very difficult to understand how a uninucleate gamete could develop such elaborate structures. But taking the suggestion of Harper (1900) that the conjugating tube of Pyronema is an outgrowth similar to a trichogyne, there are presented possibilities of various elaborate structural developments, because the outgrowth has so much protoplasm and many nuclei to draw upon. The evolutionary tendency of a cœnogamete is to reduce the number of functional gamete nuclei, generally by the sacrifice of many, but these with accompanying cytoplasm are sometimes employed to advantage in developing structural adaptations. In the Peronosporales the advantage lies in the activities displayed by the periplasm in assisting to form the spore wall. Araispora (Thaxter, 1896) utilizes the periplasm to develop a cellular envelope surrounding the egg. The conjugating tube of Pyronema is evidently a desirable specialization,

insuring a union with the male organ. Perhaps the elaborate multicellular trichogyne is the result of similar activities on the part of archicarps that are or were cœnogametes.

It is obvious that this possibility has very important relations to comparisons that have been made between the trichogynes of the Ascomycetes and those of the Rhodophycæ. It is not easy to homologize these structures and it is difficult to conceive the evolution of any group of the Ascomycetes from the red algæ. The Laboulbeniaceæ exhibit certain strong resemblances in a general similarity of cell structure, but peculiarities confront one whenever the comparison is carried into details. Nevertheless a relation of this group to the Rhodophycæ remains a possibility, although it can hardly be more than mere speculation until we have much greater cytological knowledge of sexual processes here and in other Ascomycetes.

But the cœnogamete may be found widespread among the Ascomycetes which suggests a new point of view that is worth attention. It is possible that the cœnogamete may become recognized as a primitive type of sexual organ in the Ascomycetes, as the writer believes it to be for certain regions of the Phycomycetes (Mucorales, Saprolegniales, and Peronosporales). Perhaps the complex conditions of such highly specialized groups as the Erysiphæ, lichens, and Laboulbeniaceæ may be related to the peculiar activities and possibilities of diverse development in this interesting sexual cell, the cœnogamete. Sphærotheca may readily stand as the last step in a process of nuclear reduction. Pyronema certainly exhibits the tendency to utilize superfluous nuclei and protoplasm in developing that advantageous structure the conjugating tube. And possibly such tendencies might result in the production of the elaborate trichogynes of the lichens and Laboulbeniaceæ and in the latter group the structure that resembles the procarp of the red algæ.

#### PHYLOGENY OF THE PHYCOMYCETES AND ASCOMYCETES

The reader of this paper has probably already noted that some standpoints have been taken at variance with the generally accepted ideas of relationships among the Phycomycetes and Ascomycetes, and of these groups to an algal ancestry. A protest is sure to be offered against the disregard of certain Phycomycetes and algæ in the attempt to derive the Mucorales, Saprolegniales, and Peronosporales from an ancestry with cœnogametes.

There are certain Phycomycetes much closer to the algæ than any of the groups mentioned above. Monoblepharis and Myrioblepharis (Thaxter, 1895) exhibit sexual organs, zoospores, and vegetative structure with striking resemblances in various particulars to such algæ as Vaucheria, Edogonium, and Sphæroplea. The homologies can hardly be questioned and will not be elaborated here. These fungi, and possibly some of the Leptomitaceæ, seem to be close to heterogamous (oosporic) algæ and may well have come from that region of the Thallophytes. The family Leptomitaceæ includes some very remarkable types which have been well described by Thaxter (1896). Their

position must remain somewhat uncertain until we know the nuclear structure of the sexual organs, but the general morphology of some forms indicates a relationship to the Peronosporales. *Araiospora* (Thaxter, 1896) is likely to prove especially interesting as illustrating an activity of the periplasm, in forming a cellular envelope around the oospore, that is not shown in any other type and which has important bearings on the possibilities of the cœnogamete to develop tissues of considerable complexity.

But many difficulties present themselves when the Monoblepharidæ are made a starting-point for a line of ascent to the Peronosporales, as is done by Trow (1901, pp. 306, 307) when he arranges a series Monoblepharis, Saprolegnia, Pythium, and Albugo (*Cystopus*). These forms are not so similar that close relationships are manifest either through morphology or ontogeny. The most favorable interpretation must grant that they are at present widely divergent and highly specialized types, even assuming that ancestral forms now extinct might have had more general characters. Such speculations are, of course, entirely justifiable, if they do no violence to developmental processes.

However, as has been shown, such an evolution must assume either that uninucleate gametes became multinucleate or that differentiated eggs (*Monoblepharis*) lost their high state of specialization and finally their entire individuality in the cœnogamete of the Peronosporales. Both processes are opposed to what we know has been the evolutionary history of sexual cells in several divergent and independent groups of algæ. We are called upon to accept a "subjective phylogeny" opposed to well-established cytological processes.

The situation is somewhat similar to that presented to the Brefeldian school with respect to the origin of the ascus from the sporangium of a mould. Harper has shown that the protoplasmic activities of sporogenesis in the sporangium and ascus are along entirely different lines with nothing in common. To the writer such differences in cytological processes completely outweigh conclusions from any series of types presented on a basis of general form resemblance. Form resemblance between the ascus and sporangium can have very little morphological value until it be accompanied by evidence satisfactorily explaining the differences of protoplasmic organization and behavior. And the elaborate phylogenetic structure built by Brefeld and his followers is sadly in need of a foundation, if not already a ruin. Form resemblance must be in complete sympathy with cytological conditions to have weight.

Trow (1901) has criticised a developmental line that the writer indicated in 1900, which, he states, is an attempt to derive Oomycetes from a Zygomycete-like ancestry and which he considers an example of "subjective phylogeny." I have carefully examined what was written in that paper (Davis, 1900, pp. 304-9), and, not finding any reference to specific phylogenetic ancestry, am compelled to suggest to Trow a more careful reading and citation of that article. I presented there suggestions for the developmental history of the sexual conditions in the Peronosporales from cœnogametes derived from the gametangia of algæ. These cœnogametes at a certain stage

in the process of sexual differentiation would be similar to the sexual organs of the Mucorales. The moulds were used to illustrate a well-defined sexual condition, which is not at all suggesting that they are the ancestry of the Peronosporales (Oomycetes).

But the present investigation of Saprolegnia, together with Stevens's (1901) later studies on Albugo, have strengthened my faith in the suggestions of that former paper (Davis, 1900). The Mucorales, Saprolegniales, and Peronosporales are generally acknowledged to be closely related groups, but it seems probable that the affinities are only through the somewhat similar conditions of sexual organs derived from the cœnogametes of some common ancestry. There are many peculiarities of life-habits, life-histories, and methods of asexual reproduction. Of these three groups the Mucorales presents the simplest conditions of sexuality and illustrates most nearly the structure of the primitive cœnogamete. The Peronosporales and Saprolegniales are difficult to relate to one another, for the higher development of the cœnogamete is apparently progressing along divergent lines. In the Peronosporales the protoplasmic differentiation in the oogonium determines a centrally placed egg in an enveloping periplasm, for a single cœnocentrum dominates the process of oogenesis. In the Saprolegniales the ooplasm gathers by cleavage around a number of cœnocentra, and all the protoplasm passes into the resulting eggs. To the writer the second process seems less highly specialized than the first and the Saprolegniales lower than the Peronosporales with respect to sexual processes. But oogenesis in these two groups shows such marked differences in their evolutionary tendencies that the question of the relative level of each process has very little import.

The Saprolegniales are more difficult to understand in relation to a cœnogamete ancestry than the Peronosporales, because the many eggs without periplasm suggest at once the stage in heterogamy illustrated by Sphæroplea. However, the processes of oogenesis are probably very different in the two types. The egg origins of Saprolegnia have a great many potential gamete nuclei, and that stage indicates strongly the cœnogamete ancestry. By numerical reduction of the gamete nuclei the egg of Saprolegnia has proceeded to a point where it has almost ceased to be a cœnogamete, that condition only being presented in the bi- and tri-nucleate eggs.

It will be difficult for many to give up the idea that Vaucheria is not a suitable starting-point for the line of higher Phycomycetes. The chief objection is the incompatibility of the processes of oogenesis where a relationship demands agreement even in the details of cytology. We have only the accounts of Oltmanns (1895), Behrens (1890), and Klebahn (1892), which are not in complete agreement on some important points, and perhaps further study may reveal conditions that are only suspected. In considerations of this sort it is important to know the relation that Vaucheria bears to the algæ as a whole. Although generally classed among the Siphonales, Vaucheria has little in common with that group excepting the cœnocyctic thallus. It stands alone as the only heterogamous form (oosporic) in a very large assemblage characteristically isogamous. Generally taught as a type of the Siphonales,

*Vaucheria* is not really representative of that group, which is much better illustrated by such forms as *Codium*, *Bryopsis*, or *Penicillus*. The affinities of *Cladophora* with the Siphonales are now better understood, and we see that this form, except for the septate thallus—whose cells are, however, multinucleate—has all the characters of the Siphonales. It is this region of the algæ (*Cladophora*, *Codium*, etc.) that presents to the writer's mind conditions most nearly like the ancestry of the Mucorales, Saprolegniales, and Peronosporales, that is, an ancestry whose sexual organs were cœnogametes. However, perhaps, farther studies on the oogonium of *Vaucheria* may bring this structure into sympathy with cœnogametes.

The cœnogamete among the fungi must have come through the homologous structure among the algæ, the gametangium. We cannot suppose that such gametangia were highly specialized. It is hardly possible that they were heterogamous, for a highly differentiated oogonium would not be likely to return to conditions as simple as the primitive cœnogamete. The gametangia of such insogamous algæ as *Cladophora* and *Codium* present most nearly the structure demanded of the progenitors of the primitive cœnogamete, but, of course, these forms are mentioned only as illustrations of conditions undoubtedly present in many groups of algæ at various periods in their evolutionary history.

We can only speculate as to the manner in which a gametangium might become a cœnogamete. The writer has already offered some suggestions on this point (Davis, 1900, p. 308), and he is more inclined to them since the recent studies of Harper (1900) and Stevens (1901) and the present investigation of *Saprolegnia*. We can readily conceive the derivation from isogamous algæ of groups of aquatic fungi with terminal sporangia discharging motile gametes after the manner of *Cladophora*. Should such fungi leave the water and adopt a terrestrial life either as saprophytes (Mucorales) or parasites (Peronosporales), certain changes in the sexual processes would be very likely to result. The gametangia could not form and discharge motile gametes excepting when wet, and would be compelled to adapt themselves to the aerial environment. They would be very likely to develop such unity of structure and behavior as is displayed in *Pythium* and *Peronospora* by those sporangia (conidia) which have given up the habit of forming zoospores and now germinate by a tube. The gametangium would become a cœnocyctic unit with the chemotactic qualities and possibilities associated with sexuality. These chemotactic influences might be satisfied by the fusion of the gametangia (cœnogametes) in pairs whereby the gamete nuclei would be able to unite two by two in a common protoplasmic medium. This process would take the place of the conjugation of motile gametes in water, and apparently satisfy all the hereditary demands as far as nuclei are concerned. The structure resulting from the fusion of these simplest cœnogametes would be very similar to the zygospore of the moulds.

Although there are no cœnogametes among the algæ, the sexual processes in the Conjugales have some features worth noting in this connection. In the desmids the

gametes slip from the parent cells and fuse as naked masses of protoplasm. But in the filamentous forms Zygnemaceæ and Mesocarpacæ the energids (gametes) remain in the respective parent cells which push out conjugating processes. The conjugating processes are surrounded by a cell wall so they are in every respect similar to the conjugating tube of Pyronema or the receptive papilla of the Peronosporales, excepting that they emanate from a uninucleate cell instead of a cœnocyte. It is important to note that such conjugation processes in the Phycomycetes and Ascomycetes have their analogies in the algæ, for it might be suggested that the development of such a structure by a cœnogamete would be difficult. On the contrary, it seems the natural expression of any cell, whether uninucleate or multinucleate, with chemotactic tendencies to fuse with its neighbors. It is very probable that the development of such a conjugating tube in a cœnogamete would be at the point where formerly the naked motile gametes were discharged, for that place is evidently the seat of important cytoplasmic activities.

To sum up our conception of the Phycomycetes, we must regard them as a group of several independent phyla. The Chytridiales in morphology and life-histories are the lowest and resemble the algæ at the level of the Protococcales. Monoblepharis, and probably several other isolated genera, seem most closely related to heterogamous algæ. The Entomophthorales are too highly specialized to be easily derived directly from algal ancestry and need not be considered in this paper. There are left the most conspicuous of the Phycomycetes in three orders that agree primarily in having either typical cœnogametes (Mucorales) or sexual organs probably derived from cœnogametes (Peronosporales and Saprolegniales).

These three orders can, however, only be related to one another through a common ancestry whose sexual organs were cœnogametes. The Mucorales illustrate most completely the primitive cœnogamete, and for this reason in part may be considered rather the lower of the three groups. In the Peronosporales we have an ascending series from forms such as *Albugo Bliti* and *A. Portulacæ* with true cœnogametes, although more highly specialized than those of the moulds, to the conditions in *Albugo candida*, *Peronospora*, and *Pythium*. This advance is evidently such an evolution as would provide a single uninucleate egg with the richest supply of food and best protective walls possible. In the Saprolegniales the evolutionary trend is similar in that a great many potential gamete nuclei are sacrificed to give a uninucleate egg, but we are not yet prepared to trace exactly the steps in the origin of this oogonium. However, the probabilities are that it, too, has come from a cœnogamete, and that the segmentation of this protoplasm to form many eggs does not imply a derivation from heterogamous ancestry, but special peculiarities associated perhaps with the presence of several cœnocentra. Oogenesis in Saprolegnia certainly indicates an ancestry with cœnogametes. The Mucorales, Peronosporales, and Saprolegniales then probably come from a somewhat similar ancestry with cœnogametes, which necessitates their derivation from isogamous algæ at about such a level as is illustrated today by Cladophora and forms of the isogamous Siphonales.

We do not propose to discuss the phylogeny of the Ascomycetes further than to present its problem with respect to the cœnogametes. The difficulty of relating the diverse sexual organs represented by Sphærotheca, Pyronema, the lichens, and the Laboulbeniaceæ has led to suggestions that the Ascomycetes are polyphyletic. But this view has many objections in the essential unity of the ascocarps and general rhythm of the life-histories throughout the group. Nevertheless, the various types of sexual reproduction seem very diverse when compared with one another and with conditions in the algæ and other fungi.

However, should the cœnogamete be established as a primitive type of sexual organ here as in the Phycomycetes certain difficulties will be removed. The oogonium (archicarp) would be considered a development from the cœnogamete along a well-established evolutionary line, that of numerical nuclear reduction. The evolutionary trend of the cœnogamete would then be toward the uninucleate oogonium (Sphærotheca) following the tendency of sexual evolution so well recognized in the algæ. The groups of the Discomycetes and Pyrenomycetes would then readily arrange themselves according to the structure of the ascocarp and general vegetative complexity.

There would be left the lichens and Laboulbeniaceæ, whose trichogynes at least suggest the Rhodophyceæ, while in the latter group there are certain histological resemblances to this same group of algæ. Granting these possible affinities, it is nevertheless very difficult to conceive the multicellular trichogyne as derived from the simple structure of the red algæ. It must also be borne in mind that the structure of the ascocarp, especially among the lichens, gives no suggestion of a cystocarp, but, on the contrary, presents a structure identical with the fructification of other Ascomycetes. Were it possible for the cœnogamete to develop a multicellular trichogyne (there is a multinucleate one in Pyronema), then evolutionary lines might be established that would lead very naturally into the lichens and Laboulbeniaceæ. Such trichogynes would be another form of expression of this remarkable structure, the cœnogamete, which is able to utilize superfluous protoplasm in such a variety of ways.

In this connection it is interesting to sum up the various ways in which the superfluous protoplasm of a cœnogamete may assert itself. It may form a periplasm of importance in developing the spore wall (Peronosporales). It may form a surrounding tissue from such periplasm (Araiospora). It may develop a conjugating tube (Pyronema). And finally we suggest the possibility of multicellular trichogynes derived from cœnogametes. While this cannot be more than a speculation, nevertheless cytological and developmental investigations among the lichens and Laboulbeniaceæ in relation to these possibilities are sure to bring forth interesting results.

We may then conceive the Ascomycetes as presenting two important evolutionary lines derived from a primitive cœnocyctic type of sexual organ (cœnogamete). The first, through numerical reduction of potential gamete nuclei, results in uninucleate sexual organs (Sphærotheca). The second line supposes the utilization of such potential gamete nuclei with cytoplasm to develop such secondary sexual structures as the

conjugating tube of *Pyronema* and the trichogynes and procarpic apparatus of the lichens and Laboulbeniaceæ.

It is difficult to relate the account of Juel (1902) for *Dipodascus* to conditions in other cœnogametes. Juel believes that there is but one sexual nucleus in each of these multinucleate gametes, the others being "vegetative": that there is only one fusion nucleus in the fusion cell. This gives rise to a series of nuclei around which the spores develop in the sac and the "vegetative" nuclei degenerate. The details of the nuclear activities are not reported, and many stages in the processes are completely lacking. Until we know these we must hesitate to express an opinion on the position of *Dipodascus*.

#### THE NUCLEUS OF PHYCOMYCETES IN ONTOGENY

A detailed and complete study of the nucleus of some Phycomycetes in the various phases of ontogeny is greatly to be desired. At present we know a good deal about the nuclear activities during gametogenesis and something at the time when the oospore germinates, but the data are not complete for any one form and do not explain the most important problems of ontogeny. These concern the significance of the mitoses in the gametangia, the relative numbers of chromosomes at different periods of ontogeny, and their bearing on the sequence of generations, which is not well understood in this group.

This knowledge will demand the study of one or more types with attention to nuclear phenomena during vegetative periods, especially at the time when asexual spores or conidia are formed, during gametogenesis and the mitoses following the fusion of sexual nuclei. Species of *Albugo* and *Peronospora* seem to offer the best material for these investigations. *Pythium*, although easy to cultivate and control, has nuclei so small as to be almost impossible for such details, and the same difficulties apply to the Mucorales and in part to the Saprolegniales, while in this latter group the complications of apogamy render the forms useless for these problems. Speculations on the reduction of chromosomes and the significance of various phases of ontogeny in this group are almost futile until we have convincing and complete data for one or more types.

Whatever may be the significance of the mitoses in the gametangia, there is no proof that they are reducing divisions, and it is probable that they are only phylogenetic reminiscences. Stevens's observations that the nuclei in the second mitosis of *Albugo* are much weaker in kinoplasm are interesting, but it is very questionable whether such divisions are necessary steps in the physiological differentiation of gametes. The mitosis may have simply a phylogenetic relation and the lessened kinoplasmic content be merely the result of that decrease in the size of the nuclei characteristic of advanced periods of oogenesis in these plants.

Everything seems to point to the ooplasm as trophoplasmic in character, first from the gathering of substance around the cœnocentrum, and second from the effect



of this structure on nuclei in the vicinity. Staining reactions confirm this conclusion, but it is not wise to lay too much stress on the effects of stains in objects so small as these. And for this reason the judgment that the gamete nuclei are weak in kinoplasm must be taken with caution. The nuclei are generally smaller, and the conditions are such that the majority of them must disorganize; but the reason for this run-down state must be chiefly the general nutritive conditions of the gametangium, and not the mitoses of that period.

Stevens (1901, pp. 238, 239) lays stress on that period of oogenesis in *Albugo* and *Peronospora* termed "zonation," when the nuclei often in mitosis move from the center of the oogonium to the periphery. He suggests "that the nuclei pass to the periphery to rid themselves of superfluous kinoplasm, possibly to prevent parthenogenetic development of the oosphere." This theory seems to the writer to suppose an order of events and degree of preformed specialization more intricate than the evidence warrants. It seems more likely that "zonation" represents an event that happens to accompany, but is secondary to, those processes which gather the ooplasm in the center of the oogonium and give the egg its *cœnocentrum* and characteristic alveolar structure.

Indeed, the conditions that cause that extraordinary degeneration of nuclei in the oogonium must furnish in large part the solutions of these problems. This phenomenon is universal whether the nuclei break down in the eggs themselves (*Saprolegniales*) or are relegated to such secondary sexual structures as periplasm (*Peronosporales*) or a conjugating tube, as in the Ascomycete *Pyronema*. As we have seen in *Saprolegnia*, the many nuclei in the eggs during advanced stages of oogenesis are all much reduced in size, and the only thing that saves the fortunate survivors of the generally severe conditions is proximity to that center of metabolic activity, the *cœnocentrum*. There is a limit to the number of nuclei possible in a given amount of cytoplasm. The nutrition of the oogonium decreases as oogenesis proceeds, and finally reaches a point when the nuclei are sorely pressed to maintain themselves. This certainly seems to be the history for the *Saprolegniales*, and probably every *Phycomycete* whose sexual organs are *cœnogametes*, as these structures are generally formed late in ontogeny when the period of vegetation is about completed.

The nuclei are then subjected to a keen struggle for existence, and, in spite of the fact that they are in a symplast, which is itself a unit, they may well be supposed each to look after its own interest as far as possible. The outcome of that struggle is largely determined by the activities of the cytoplasm, which may develop such metabolic centers (morphologically expressed by *cœnocentra*) that certain nuclei by good fortune of favorable position are given great advantages over their neighbors and finally selected as the survivors.

There are a number of instances known where structures sacrifice some of their nuclei to provide the remainder with the cytoplasm at hand. Certain of the *Fucales* are notable examples, and there will probably be found other illustrations among the

algæ and fungi. Analogous conditions in animals have been reported, as in oogenesis of *Actinosphæria* (Hertwig, 1898), and the well-known fate of supernumerary male nuclei in polyspermy. In these cases there has not been reported the same close relation between the surviving nuclei and metabolic centers of the cell as between the favored nuclei and the cœnocentra of the Saprolegniales and Peronosporales. In this same connection we need more detailed accounts of oogenesis in the Fucales, *Vaucheria* and *Sphæroplea*.

The reasons why the oogonium overstocks itself with nuclei are probably phylogenetic and recall the time when numerous uninucleate gametes were formed from the protoplasm that now acts as a unit (cœnogamete). Such uninucleate gametes were probably smaller than their homologues, the asexual zoospores, as is so characteristic of algæ. Among the algæ it is generally conceded that the small gamete swarm spores result from different conditions of nourishment than their asexual homologues. It has been suggested that they are starved, but that seems a clumsy conception of very intricate processes. But there must be deep significance in the overproduction of sexual nuclei during gametogenesis and its obvious association with the deficient nutrition at the command of the gametangia. This phase of the subject has not received the attention it deserves.

#### SUMMARY OF THE INVESTIGATION OF SAPROLEGNIA

##### OOGENESIS

The material, *Saprolegnia mixta*, was apogamous, being entirely free from antheridial filaments.

The resting nucleus has a loose linin network and a nucleolus, and presents essentially the structure of the nucleus of higher plants.

There is one mitosis in the oogonium, the spindle being intranuclear. There are no centrosomes. The four chromosomes are derived from the linin network.

The daughter-nuclei following the mitosis are much smaller than their parents. They shortly give evidence of coming degeneration, the nuclear membranes become indistinct, and the contents finally lie as granular material in clear areas resembling vacuoles.

The eggs are formed during the process of nuclear degeneration. The protoplasm in the oogonium at this period is arranged peripherally around a large central vacuole. The ooplasm collects around several centers, each of which is to become an egg origin. The egg origins are finally separated through the arrangement of vacuoles which results in the severance of connecting strands of protoplasm, and the eggs round themselves off as independent structures.

The differentiation of the egg origins takes place around a deeply stained protoplasmic body, the cœnocentrum, from which delicate fibrillæ radiate. The cœnocentrum is formed *de novo*, one for each spore origin. It is at first a small globule, made conspicuous, however, by its fibrillar rays. It is most conspicuous in the young eggs,

becoming less distinct with the ripening, and finally disappears. There is no trace of it in the oldest eggs.

The cœnocentrum is a protoplasmic structure, but not a permanent organ of the cell. It is probably the morphological expression of dynamic activities in the oogonium when the egg origins are differentiated, and is a sort of focal point of the metabolic processes peculiar to oogenesis.

The cœnocentrum exerts a chemotactic influence on any nuclei in its immediate vicinity. Generally one nucleus is selected and comes to lie very close to the cœnocentrum, so that these two structures in the egg origins may be separated only under high magnification. This nucleus increases in size when all other nuclei in the egg origins and young eggs are degenerating, showing that it is greatly favored with respect to nourishment by its position near the cœnocentrum.

Sometimes two or even three nuclei may lie sufficiently near the cœnocentrum to be saved from degeneration, and such eggs are in consequence bi- or trinucleate. Binucleate eggs are not uncommon, trinucleate eggs are more rare.

As the eggs mature, the favored nucleus increases greatly in size, until it is many times larger than at the period following the mitosis. The other nuclei have generally completely disorganized, but sometimes traces remain as granules scattered in the cytoplasm.

Binucleate eggs in the Saprolegniales need have no relation to the problem of sexuality, and Trow's conclusions are not established.

#### SPOROGENESIS

A general confirmation of the accounts of Rothert, Hartog, and Humphrey.

The uninucleate spore origins are differentiated by clefts that push their way from the central vacuole of the sporangium to the periphery. When the clefts reach the cell wall, the turgor of the sporangium is relieved through the escape of water, and the spore origins run together, but they soon draw apart and round themselves off as zoospores. There seem to be no cytoplasmic centers in the sporangium comparable to the cœnocentra.

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### EXPLANATION OF PLATES

The material, fixed in weak chrom-acetic acid, was cut  $3\mu$  thick and stained on the slide with safranin and gentian violet. All figures were sketched with an Abbe camera under the Zeiss apochromatic objective 2 mm. aper. 1.30, or 1.5 mm. in combination with compensating oculars. The magnification is as follows: Fig. 1, 250 diameters; Figs. 2-5, 12, and 13, 500 diameters; Figs. 6-9, 1,500 diameters; Figs. 10, 11, and 14-29, 1,000 diameters; Figs. 30-35, 667 diameters.

#### PLATE XV

(Figs. 1-15 illustrate Oogenesis)

- FIG. 1.—End of hypha about to form an oogonium.  
 FIG. 2.—Young oogonium, nuclei approaching spirem.  
 FIG. 3.—Central vacuole forming.  
 FIG. 4.—Central vacuole; nuclei in spirem.  
 FIG. 5.—More advanced than Fig. 4; nuclei in mitosis.  
 FIG. 6.—Details of nucleus in spirem condition.  
 FIG. 7.—Metaphase of mitosis; spindle intranuclear; nucleolus outside of spindle; three chromosomes shown.  
 FIG. 8.—Mitosis just after the splitting of chromosomes at nuclear plate; nucleolus outside the spindle.  
 FIG. 9.—Anaphase; two groups of chromosomes, four in each group, at the poles.  
 FIG. 10.—Oogonium after mitosis with twice as many nuclei as previous to that event.  
 FIG. 11.—Oogonium older than in Fig. 10; nuclei degenerating.  
 FIG. 12.—Formation of egg origins under low magnification (500 diameters); coenocentrum in center of each egg origin.  
 FIG. 13.—Egg origins older than in Fig. 12; coenocentra with conspicuous radiations.  
 FIG. 14.—Coenocentrum before the differentiation of the egg origins; radiations plain; nucleus at one side of coenocentrum; other nuclei degenerating in the cytoplasm.  
 FIG. 15.—Similar to Fig. 14; two coenocentra; nucleus at the side of each; many nuclei degenerating in the cytoplasm.

#### PLATE XVI

(Figs. 16-29 illustrate Oogenesis)

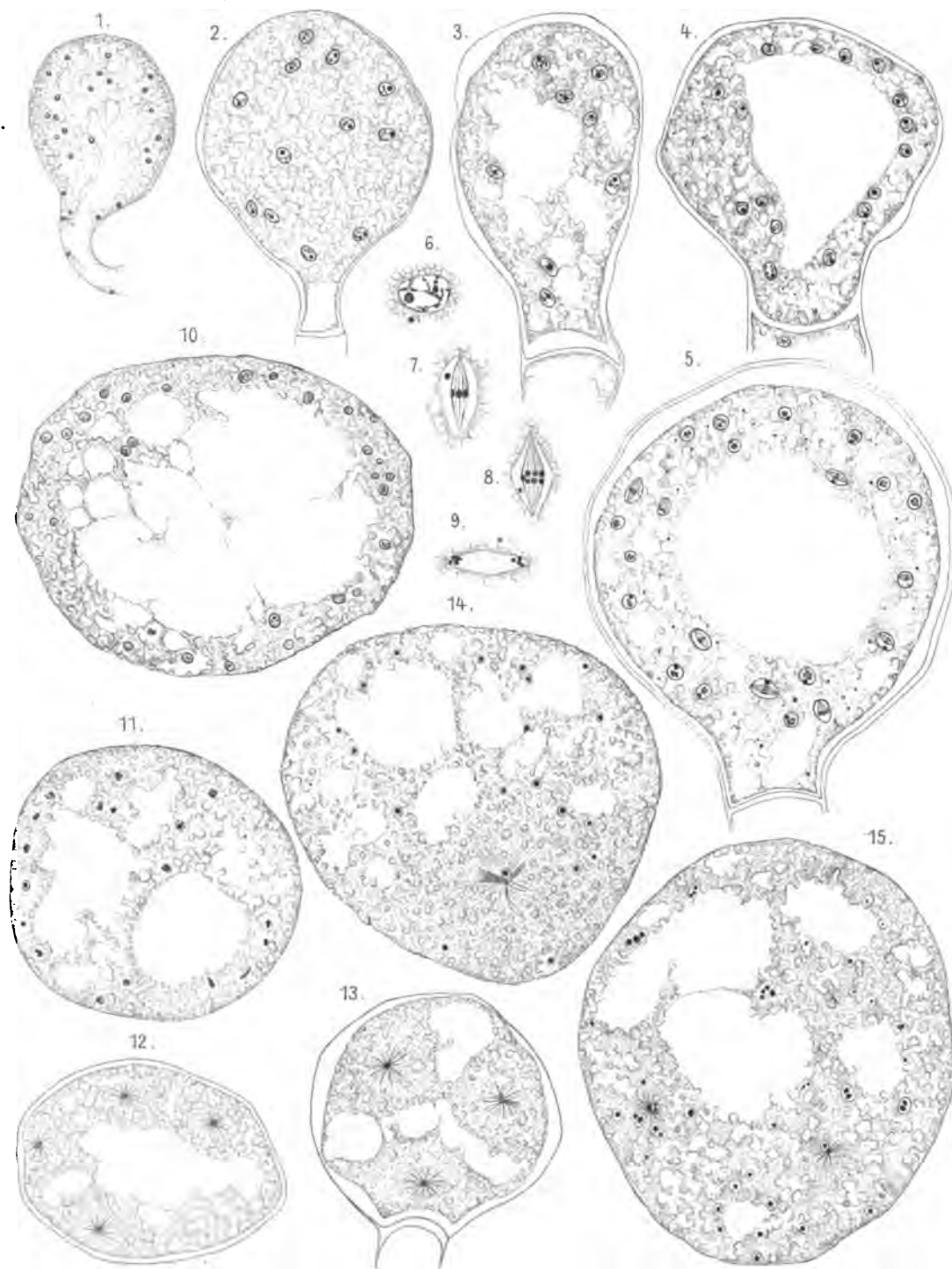
- FIG. 16.—Egg origin just before rounding off to form egg; conspicuous coenocentrum with nucleus at the side.  
 FIG. 17.—Young egg, nucleus larger than in Fig. 16.  
 FIG. 18.—Young egg; coenocentrum without radiations.  
 FIG. 19.—Egg, older than in Figs. 17 and 18; nucleus larger.  
 FIG. 20.—Egg with nucleus extended toward coenocentrum, which has almost disappeared.  
 FIG. 21.—Mature egg; large nucleus; coenocentrum disappeared.  
 FIG. 22.—Young binucleate egg, the two small nuclei close to the smaller coenocentrum.

- FIG. 23.—Young egg with two coenocentra, each accompanied by a nucleus.  
FIG. 24.—An exceptionally large binucleate egg with prominent coenocentrum.  
FIG. 25.—Egg with two nuclei lying over one another, both extended toward the coenocentrum.  
FIG. 26.—Binucleate egg with the nuclei at a distance from one another.  
FIG. 27.—Binucleate egg with the nuclei close together.  
FIG. 28.—Trinucleate egg, the three nuclei lying close together.  
FIG. 29.—Trinucleate egg, the three nuclei at a distance from one another.

(Figs. 30–35 illustrate Sporogenesis)

- FIG. 30.—End of sporangium showing development of central vacuole.  
FIG. 31.—Portion of cross section of sporangium, central vacuole well developed.  
FIG. 32.—Early stage of segmentation; cleavage furrows running from central vacuole to periphery.  
FIG. 33.—After cleavage furrows have reached periphery, spore origins forming.  
FIG. 34.—Spore origins older than in Fig. 33.  
FIG. 35.—Zoospores in sporangium.



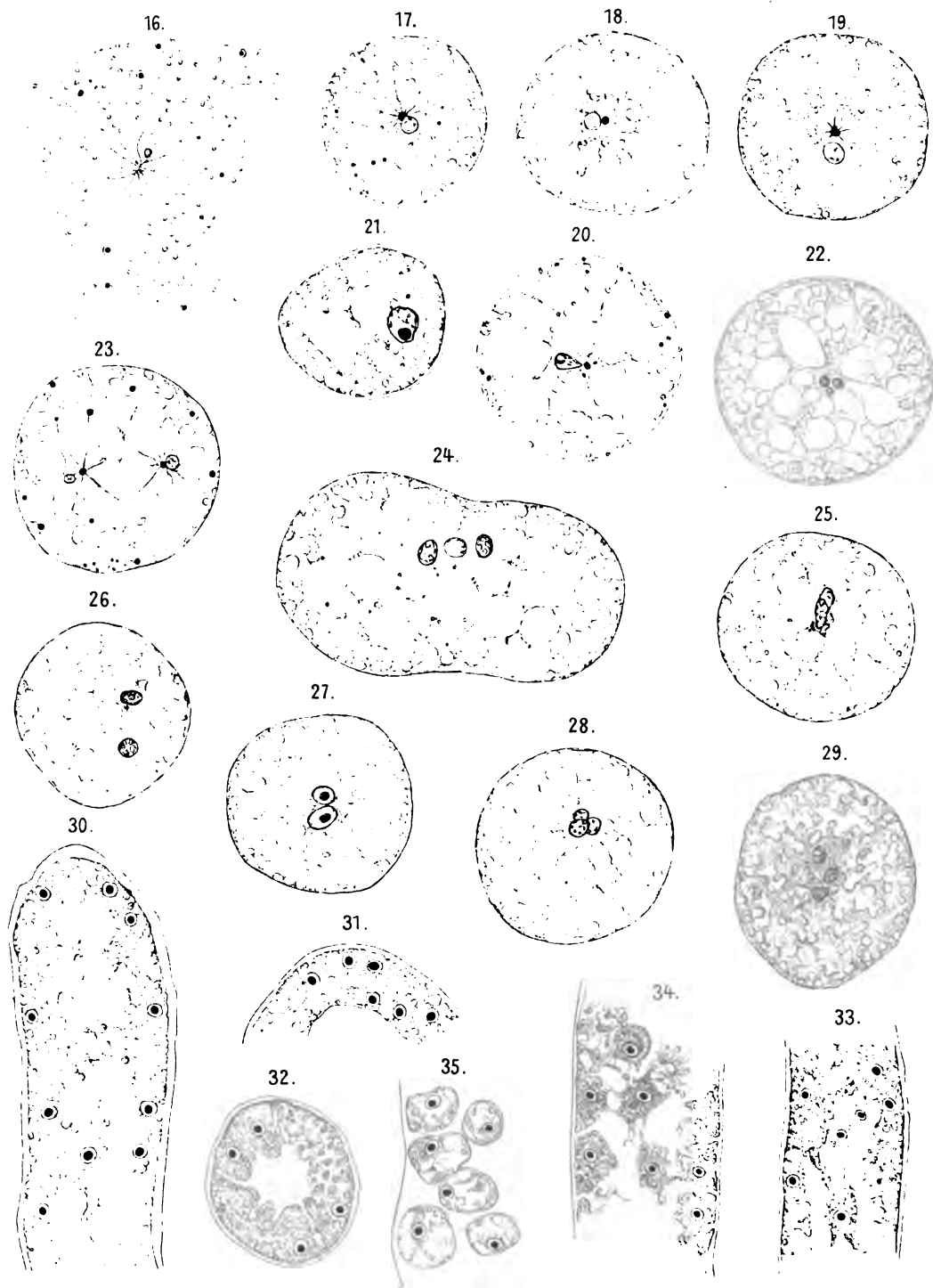


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**THE EARLY DEVELOPMENT OF LEPIDOSTEUS  
OSSEUS**



# THE EARLY DEVELOPMENT OF LEPIDOSTEUS OSSEUS

ALBERT CHAUNCEY EYCLESBYMER

## INTRODUCTORY

CERTAIN phases of the early development of *Lepidosteus* have been described and depicted by Balfour and Parker, Beard, Dean, and others. These investigators studied preserved material only, and arrived at widely different conclusions regarding certain fundamental features of development. Balfour and Parker (1882) and Beard (1889) consider the egg as holoblastic, while Dean (1895) maintains that it is meroblastic. Thus it is evident that either the egg of *Lepidosteus* is quite unlike that of any other known vertebrate, or that the character of its cleavage has been misinterpreted.

In 1897 the writer obtained an abundance of the embryological material and found that no one had described the cleavage as presented by the living egg; attention has been called to this fact in a preliminary note (1899). The detailed study of the preserved material not only has confirmed the deductions made, but also has revealed other new features of development, which will be described in the following pages.

The greater part of the material for this study was obtained June 13, 1897, from La Belle Lake, near Oconomowoc, Wis. Some material was obtained June 20, 1900, from Sand Lake, near Nottawa, Mich. The fish were taken by spearing and, although more or less mangled, the eggs and sperm were in most cases in such an excellent condition that artificial fertilization was easily produced. The eggs and sperm obtained by pressure ("stripping") were more favorable for fertilization than when got by excision. The eggs were fertilized in earthen dishes, in which they remained until the embryos hatched.

From this material several series were preserved, comprising the stages between the unsegmented egg and the 25 mm. larva. In preserving the eggs the following fixing fluids were used: formaline in 8 to 10 per cent. aqueous solution, which, followed by hæm-alum surface-staining, clearly defined the cleavage grooves; chrom-acetic, to which a little 1 per cent. osmic acid was added, gave most excellent results; corrosive sublimate, saturated aqueous solution, plus 1 per cent. glacial acetic acid, proved to be most satisfactory for sectioning and for section-staining; picro-acetic and picro-sulphuric, while fairly good for the larval stages, caused swelling and distortion of the eggs often entirely obscuring the cleavage grooves.

Since the writer's observations on the general and breeding habits of *Lepidosteus* add nothing of importance to the records of other observers, the study may be introduced by a general description of the surface changes going on between the freshly fertilized egg and the larva at the time of hatching.

## OUTLINE OF DEVELOPMENTAL PHASES UP TO TIME OF HATCHING AT 20° C.

In nearly all the eggs the first cleavage begins about one and one-half to two hours after fertilization. At the end of six hours a cap containing 250-300 cells has been formed. Four hours later the blastodermic cap extends downward over a distance equal to one-third the long axis of the egg. About the time the rim of the extending blastodisc reaches the region of the equator, a faint linear tract grows out from the margin of the blastodisc toward the upper pole. This tract, which is lighter than the surrounding parts, represents the *Anlage* of the forthcoming embryo. At the end of twenty-four hours the blastopore is reduced to about one-third the diameter of the egg, and four hours later is barely visible; the embryo meantime becomes well defined. In from forty-five to forty-eight hours after fertilization faint lateral swellings mark the beginnings of the optic vesicles, neither the cephalic nor the caudal end of the embryo is as yet free from the yolk, nor is there any trace of the pronephric ducts. In about fifty-six hours the embryo has reached a length of about  $2\frac{1}{2}$  mm.; the cerebral vesicles are plainly marked, the mid-brain being especially distinct; the bases of the adhesive disks are discernible; the tail of the embryo is becoming free from the yolk, while the pronephric ducts and mid-dorsal myotomes are visible. About eighty hours after fertilization the first movements of the larva are observed; these movements are but momentary, and are confined to the anterior mid-dorsal myotomes. Sections of the embryos of this period show that the appearance of these movements is practically coincident with the differentiation of fibrillæ in the myoblasts. The envelopes at this time measure 4-5 mm. in diameter, leaving a considerable space around the larva. During the fourth day the larva attains a length of 6-7 mm. When freed from the envelopes it strives to maintain an upright position; if it be accidentally turned on its side, it makes a few brisk movements and attains the position sought. It is of interest to note that the larval *Amia* of a corresponding stage is wholly unable to maintain or regain an upright position. On the fifth day the larva has reached a length of 8-9 mm. and is more active. If the envelopes be removed, it makes vigorous efforts to swim about, but is barely able to sustain its heavy load of food-yolk. The adhesive disks are now functional, and the larva clings to various objects, often so tenaciously that they are not detached by the action of the killing fluids.

Sometime during the sixth day the larva breaks through the surrounding envelopes, which have now become soft and flimsy, and swims vigorously about, rarely coming to rest until some object is found to which it can easily adhere. It is noticeable that the larva is much stronger and more active than the larva of *Amia* at a corresponding stage, being able to support and easily move its mass of food-yolk. At this time the yolk-sac has elongated and become slightly narrowed at its middle. The beginnings of the operculæ can be distinguished. Pigment is present on the upper surface of the yolk; it is likewise scattered over the head, along the sides of the body, and faint traces are seen in the retinæ.

The observations made by different investigators indicate that the time of hatching varies from three to nine days, as shown in the following table:

Authority	No. Days, Fertiliza- tion to Hatching
Garman and Agassiz - - - - -	8
Mark - - - - -	7
Beard - - - - -	9
Beard - - - - -	7
Dean - - - - -	8
Perry (per Dean) - - - - -	5
Fülleborn - - - - -	3
Eycleshymer - - - - -	6

It is a significant fact that the longest periods indicated were those in which the eggs were laid during the month of May, while in the other cases cited the eggs were laid during the month of June. It is therefore probable that the explanation of this variation is to be found, in part, at least, in the differences in water temperature.

A description of the changes which the larva undergoes after hatching would be of little value in the present paper, but for the convenience of those who may obtain isolated stages a brief tabulation of some of the more conspicuous changes is here given:

Age in Hours	Length in mm.	Stage of Development
0	.....	Fertilization, beginning of
1.40	.....	First cleavage, beginning of
2.15	.....	Second cleavage, beginning of
2.48	.....	Third cleavage, beginning of
3.25	.....	Fourth cleavage, beginning of
4.00	.....	Fifth cleavage, beginning of
4.36	.....	Sixth cleavage, beginning of
5.10	.....	Late cleavage, 100 to 200 cells
5.45	.....	Blastula, 200 to 300 cells
10.00	.....	Gastrulation, beginning of
18.00	.....	Blastodisc extends over one-third diameter of egg
24.00	.....	Embryo appears, blastopore one-third diameter of egg
28.00	.....	Blastopore closes
45.00	.....	Optic vesicles distinguishable
80.00	4-5	Embryo begins to move
145.00	9-10	Embryo hatches
185.00	10-11	Pectoral fins appear
312.00	13-14	Pelvic fins appear
480.00	21-22	Yolk absorbed

#### CLEAVAGE

THE term "cleavage," as here used, covers the period intervening between the formation of the first cleavage groove and the beginning of gastrulation. The description which follows is based upon a detailed examination of the surface phenomena as presented in both living and preserved material, together with a study of serial sections of corresponding stages.

Immediately after fertilization the villi of the external envelope of the egg become



attached at their peripheral ends to the first object touched; the egg is thus supported in a fixed position until the larva escapes. The egg is oval in profile like that of *Amia*, although a trifle larger, measuring in its longest diameter, including membranes, 3–3.5 mm.; in its shortest, 2.5–2.75 mm. The upper portion of the egg, for which the term “calotte” seems preferable, is a pale creamy gray, which shades off, in a zone midway between the upper pole and the equator, into the pale drab of the yolk, as indicated in Fig. 1 (Plate XVII). Although the calotte usually appears circular in outline when viewed from the upper pole, as in Figs. 11–20 (Plate XVII), it is sometimes oval and frequently irregular. No features of contour or pigmentation have been detected which would enable one to predict the direction of any of the early cleavage grooves, or the direction of the future embryonic axes. Near the center of the calotte a single micropylar orifice is present, as described by Mark, Dean, and others. Its position with reference to the cleavage grooves is variable.

A vertical section of a mature ovarian egg is represented in Fig. 30, which shows the extent and relation of the finely granular germinal part to the coarsely granular nutritive portion. It is to be especially emphasized that this condition is a most peculiar one and, so far as the writer is aware, does not occur elsewhere among the Ichthyopsida. In this egg there is added to the blastodisc or calotte of the Teleostean egg a conical prolongation which extends to, or beyond, the equator of the egg, giving to the finely granular portion a pear-shaped outline. Around the margin of this conical prolongation are scattered numerous small vesicular structures, as shown in Figs. 30, 31, 32, 34.

I have carefully and repeatedly read the descriptions given by the various writers, but have been unable, save in a single instance, to find any reference to this strikingly peculiar condition. In Plate V, Fig. 1, of Mark's (1890) illustrations a section of the ovarian egg is represented which suggests that he observed something of the same sort. Concerning its significance nothing is said beyond the brief reference given in the explanation of the figures, which reads as follows: “The finely granular and vacuolated portion of the yolk beneath the germinative vesicle is in the center of the egg.”

*First cleavage.*—The first cleavage, as in *Amia*, is foreshadowed by a flattening of the upper pole of the egg, which in reality is a thinning of the calotte at its center. This soon changes to a wide shallow furrow, which in turn gives rise to a narrow deep fissure or cleavage groove. The groove soon extends in opposite directions over the surface of the egg, giving rise to the two blastomeres as shown in Figs. 2, 11. Its progress through the calotte is rapid and continuous, requiring but five to eight minutes to traverse the distance from center to periphery; beyond the margin of the calotte the groove travels at a gradually decreasing rate, as shown in Figs. 2–5 and 11–15, until it reaches a zone lying somewhat below the equator, where it either fades out or terminates abruptly. In most cases it seems to reach its maximum extension at the time of the sixth or seventh cleavage. Its extent seems to be fairly constant, and in no case

under my observation have the ends of this groove extended to the lower pole or even to its vicinity. Neither have I ever found them terminating in or near the margin of the calotte. Variations in the formation of this furrow are seldom observed. Among fifty preserved eggs but seven were found in which the first two blastomeres showed marked difference in size. In no case has the writer observed the pronounced inequality which so frequently occurs in the cleavage of the egg of the Teleost (*Coregonus*, *Serranus*, *Amiurus*).

A vertical section of an egg just before the appearance of the second cleavage is shown in Fig. 32 (Plate XVIII). The line of the section is indicated by the dotted meridional line in Fig. 21. The groove has cut deeply into the germinal area at its center, but as it passes toward the margin it decreases in depth, as shown in Fig. 33 (Plate XVIII), which is taken along the lower dotted line of Fig. 21. The blastodisc has undergone no visible changes in form or extent. The plane of the dividing nuclei is indicated in the figure; it will be observed that these nuclei lie well up toward the surface of the blastodisc.

Dean has described a section of an egg in a stage slightly earlier. Of this he writes as follows: "Sections, however, indicate that at this stage germ-disc and yolk are more intimately connected, and in Plate II, Fig. 21, it will be seen that the protoplasm of the animal pole is hardly to be separated from the coarsely granular yolk until nearly in the equatorial region of the egg. In the figure the nucleus is seen dividing at about one-third the diameter of the egg from the surface." The first reading of the above led me to believe that, if the cleavage grooves originated at the point shown in Dean's Fig. 21, Plate II, they must either become completely obliterated centrally, or extend much deeper than Dean had described for the succeeding stages. Moreover, it was at first inferred that Dean had observed and described the peculiar conical elongation of the blastodisc. A careful examination of the drawing of the section Fig. 21, Plate II, upon which Dean based the above statement, plainly reveals the fact that, instead of its being a vertical section, as stated by the author, it is cut in an oblique plane, thereby giving the appearance described. In the next figure (Fig. 22) given by Dean the position of the nuclei is more accurately represented, and it is, moreover, apparent that the peculiar form of the blastodisc has been overlooked.

*Second cleavage.*—This cleavage begins about thirty minutes after the appearance of the first. It is always foretold by a rapid closing of the first cleavage groove. The two grooves of this cleavage usually begin at the same point and extend in opposite directions, thus giving rise to an apparently continuous groove. In the egg followed in detail (Fig. 3) the groove on one side arose slightly in advance of that on the opposite side, as indicated by the times affixed. The furrows progress at the same rate as the first, passing from the center to the margin of the blastodisc in a few minutes; beyond the margin their progress is greatly retarded, and not until several hours later do the grooves reach their maximum extension, finally terminating in a zone near the equator. Variations in the position of the grooves sometimes occur; instead of

forming right angles with the first they form acute or obtuse angles, thereby giving rise to decidedly unequal blastomeres. Again, the grooves, instead of passing in meridional planes, may converge toward the first furrow, but rarely is this convergence so pronounced that the two furrows fuse. The variations are more numerous than in *Amia*, but far less frequent than in the Teleosts (*Amiurus*, *Serranus*, *Coregonus*). A shifting of the blastomeres is frequently observed, although not to such an extent as in the Amphibia (*Necturus*, *Amblystoma*, *Diemyctylus*, *Rana*, *Bufo*, *Acris*).

Sections show the depth of these furrows to be about the same as that of the first. It is a noticeable fact that the cleavage grooves, instead of following a given plane, become more or less tortuous, as shown in Fig. 34 (Plate XVIII). An intercellular space is frequently present at the line of intersection of the first and second cleavage grooves. From the position of the elongated nuclei of the four-cell stage one can predict the planes of the ensuing divisions. In all cases in which the horizontal sections passed through the nuclei, the latter were as indicated in Fig. 25 (Plate XVIII).

Balfour and Parker figure these grooves as extending to the immediate vicinity of the lower pole. The description by Beard leads to the conclusion that they actually reach the lower pole. Dean states that this cleavage "is expressed in the germ-disc only, and like the former furrow could not be traced into the yolk region of the egg." Among all the eggs which I have examined I have never found one in which these furrows extended as far as described by Balfour and Parker, neither have I observed the condition described by Dean.

*Third cleavage.*—The four grooves which constitute this cleavage are, in all cases examined, vertical; they usually occupy the positions shown in Figs. 4, 19, 20 (Plate XVII), conforming in general to the pattern of the Teleost. Sometimes, however, one observes the condition shown in Fig. 13 (Plate XVII), where the grooves approach a meridional position. The order of their appearance is irregular, beginning in this or that quadrant and without sequence, only a few minutes elapsing between the origin of the first and the last. In the egg followed in detail (Fig. 4), the times at which these furrows form are indicated by the numerals affixed. They travel at the same rate as those of the second cleavage, and finally terminate at about the same latitude.

Three horizontal sections of a blastodisc at this stage are represented in Figs. 26, 27, 28 (Plate XVIII). The sections are taken at different levels, and show the extent of the cleavage grooves long before they are apparent on the surface. It will be noticed that the intercellular space has enlarged. In other eggs much larger spaces are sometimes found. While as yet there is nothing which could be called a cleavage cavity, it nevertheless seems probable that this space may be its forerunner, or at least that it later contributes to its formation. It is worthy of remark that in *Amia* the cleavage cavity takes its origin in an entirely different manner, being formed by the union of a number of spaces which are present in the egg as early as the first cleavage. Fig. 34 (Plate XVIII) represents a vertical section along the plane of the dotted line in Fig. 23; it shows the relative depth of the furrows at this time. The

first groove has progressed toward the center of the egg, but a comparison with Fig. 32 shows that its rate after reaching the depth shown in Fig. 32 is exceedingly slow.

Concerning the extent of the grooves of this cleavage, authors are again at variance. Beard believes that they extend to the lower pole like those of the first and second cleavage. Dean says, however, that "in depth and lateral extension its furrows are entirely similar to those of earlier cleavages."

*Fourth cleavage.*—The fourth cleavage is, as stated by Dean, "again a vertical one." The eight grooves forming this cleavage usually occupy the position shown in Fig. 20 (Plate XVII). In all the eggs examined but few were found which did not in general conform to this pattern. These few showed variations which resemble Fig. 5 or Fig. 19. The furrows of this cleavage extend to the equator of the egg and here fade out. In the egg shown in Fig. 5 the times and points of origin of some of these grooves are recorded.

Although, as stated, variations are sometimes found, it is of interest to note that these are far less numerous than those observed in *Amia* at a corresponding stage. Again, the fourth cleavage of *Amia* is usually circular, while that of *Lepidosteus* is most frequently vertical, the latter showing a tendency toward the Teleostean pattern. This cleavage in *Acipenser*, *Amia*, and *Lepidosteus* marks an interesting phase of the development, since it is the first to result in the formation of small cells at the active pole of the egg. A vertical section of this stage is shown in Fig. 35 (Plate XVIII), its plane being shown by the dotted line in diagram 24. The direction of the ensuing cleavages is indicated by the elongated nuclei.

*Fifth cleavage.*—With the formation of the fifth cleavage interesting changes are introduced. It comprises two distinct sets of grooves. The first set appears on the surface, as shown in Fig. 15 (Plate XVII), recalling the conditions observed in *Amia*. Often the grooves pass as shown in Fig. 15, in which case they give rise to a circular cleavage. More frequently it is of a mixed character, some grooves passing in vertical planes. The second set is confined to the four central cells and is not apparent on the surface; these four cells are divided in horizontal planes, as indicated in Figs. 35, 36 (Plate XVIII). The reconstruction of an egg in this stage gives Fig. 29, in which the elongated nuclei indicate the planes of the grooves constituting this cleavage. The variations from this pattern are numerous, and no attempt has been made to record them. If one were to offer a type, it would be that given in Fig. 29.

If this cleavage of *Lepidosteus* be compared with that of *Amia*, on the one hand, and the Teleost, on the other, some interesting facts are revealed. In order to make the comparison lucid we must recall that the fourth cleavage of *Amia* is usually circular. Whitman and Eycleshymer, speaking of this cleavage, state that "it represents, strictly speaking, eight distinct grooves, one for each of the primary segments, but these usually run together in such a way as to form a continuous groove, encircling the pole and bounding a polar field which may have a circular or oval form. This circular groove looks externally as if it might actually cut off polar segments, but, as

an examination of sections will show, the groove descends more or less obliquely, or centripetally, so that when completed its inner deeper portion would be lost in confluent vacuolar spaces." The fifth cleavage, then, in *Amia* cuts off eight cells; that of *Lepidosteus*, four; that of the typical Teleost, four. This fact is emphasized because it is another point in the ontogeny of *Lepidosteus* which shows a closer resemblance to the Teleost than does *Amia*.

*Later cleavage.*—The next cleavage, which might be designated as the sixth, divides the eight central and the twenty-four marginal cells. The divisions are in general vertical, but many variations appear. I am able to confirm the statement of Dean that "the only cell divisions which were noted as generally constant were those of the marginal cells." Dean's statement that "its furrows extend no further than the margin of the cell cap" is not confirmed. Indeed, these furrows extend well down into the equatorial region, as depicted in Figs. 8–10. Vertical sections of these stages are represented in Figs. 37, 38; the former is taken from the margin of the blastodisc, while the latter passes through its center. The blastodisc is now in general two layers of cells deep, although some of the cells are now entering upon the seventh cleavage, as indicated by the horizontal division of one of the cells in the lower row. The segmentation cavity is poorly defined, being represented merely by intercellular spaces. The periblastic nuclei are most numerous at the center and are here undergoing active division, contributing one derivative to the cell cap, quite unlike that of the Teleost, but similar to the process in *Amia*.

After the sixth cleavage no attempt was made to follow the succession of grooves as presented by the living egg. The detailed study of the later stages is exceedingly difficult, owing to the fact that as cleavage goes on its character, as a whole, is somewhat modified. In the earlier stages of growth there are distinct periods of rest and activity; in the later stages this rhythm is gradually lost and the egg passes from a stage of rhythmical growth to that of continuous growth. As to the extent of the marginal grooves in the later stages, it may be said that they always terminate in an equatorial zone as depicted in the various figures. In Figs. 9, 10, 16, 17, 18 (Plate XVII), two series of later stages are represented. The eggs examined in these stages were from three different lots of preserved material and comprised several hundreds, yet in all this material there was never found an egg which conformed to the descriptions or illustrations given by Balfour and Parker, Beard, or Dean.

In the later stages of cleavage one frequently observes that the cells at one side, or margin, of the blastodisc are much smaller than those on the opposite side. Such conditions were found in the eggs represented in Figs. 16, 17, 18. While there is no question as to the existence in many eggs of areas of accelerated cell-division, I have been unable to determine whether or not these areas bear a definite and fixed relation to the embryo.

The section represented in Fig. 39 (Plate XVIII) is taken in a meridional plane through the blastodisc of an egg in the stage shown in Fig. 8 (Plate XVII). It will be seen that another set of horizontals has considerably increased the thickness of the cell

cap, which is as yet made up of loosely scattered cells. A number of the intercellular spaces in this particular egg have become confluent below the body of the cell cap, thereby giving rise to a fairly well-defined segmentation cavity. The periblast forming the floor of the cavity continues to bud off cells, not only from its margin, as in the Teleosts, but irregularly over its entire surface. In none of the eggs at this stage has the writer found the "entire floor to be smoothly and distinctly differentiated," as stated by Dean. In Fig. 40 a transverse section of this same stage is shown; the section passes in a horizontal plane, midway between the equator and the upper pole, and shows the depth of the cleavage grooves at this period. The vertical section shown in Fig. 41 (Plate XVIII) is taken from an egg in the stage shown in Figs. 9, 18 (Plate XVII). Few noteworthy changes have occurred other than the increase in thickness of the cell cap.

In Fig. 42 a section is shown from an egg in the stage represented by Fig. 10. The plane of section is intermediate between horizontal and vertical, and shows an apparent shortening of the conical portion of the periblast. When contrasted with the preceding section, one notes that the cell cap has become more disc-like, owing to its marginal expansion over the yolk. The cells constituting the body of the cell cap are small, spherical, and fairly uniform. Its outermost layer is now transformed into a layer of elongated cells which form the superficial epiblast. The lower layer is made up of yolk-laden cells which are scattered loosely in the irregular segmentation cavity. The floor of the segmentation cavity must be regarded as periblast plus its yolk-laden derivatives. Fig. 43 (Plate XVIII) is a diagrammatic representation of a transverse section in the equatorial region showing the depth and distribution of the cleavage grooves.

Throughout the stages thus far studied the peculiar conical prolongation of the periblast has undergone little or no change in either form or structure. It is therefore premature to speculate as to its significance. A study of the later stages will undoubtedly throw some light upon the function of this hitherto unknown structure.

#### SOME GENERAL REMARKS

Balfour and Parker first observed, described, and figured the cleavage of *Lepidosteus*. Their conception of its character is concisely stated in these words:

We have observed several stages in the segmentation, which show that it is complete, but that it approaches the meroblastic type more nearly than in the case of any other known holoblastic ovum.

Our earliest stage showed a vertical furrow at the upper or animal pole, extending through about one-fifth of the circumference, and in a slightly later stage we found a similar furrow at right angles to the first. We have not been fortunate enough to observe the next phases of the segmentation, but on the second day after impregnation the animal pole is completely divided into small segments, which form a disc, homologous to the blastoderm of meroblastic ova; while the vegetative pole, which subsequently forms a large yolk-sac, is divided by a few vertical furrows, four of which nearly meet at the pole opposite the blastoderm. The majority of the vertical furrows extend only a short way from the edge of the small spheres, and are partially intercepted by imperfect equatorial furrows.

Beard next described the cleavage phases stating that "the segmentation is very unequal, but in a sense complete. Eight furrows can be traced to the center of the lower pole. The attempt to segment the lower hemisphere is, however, soon given up, none of the eight furrows penetrate very deeply into the yolk, and none reach the center by a long way. They are only superficial furrows."

Dean more recently gives a description and delineation of the successive cleavages, and concludes that the egg is meroblastic. In the detailed accounts of the successive meridional and vertical cleavage grooves (pp. 16-19) he explicitly states and repeats that the grooves extend no farther than the margin of the blastodisc.

Beard still later affirms his former statement and adds these words:

If Dean were correct, the segmentation in *Lepidosteus* would be to all intents and purposes that of *Scyllium*, whereas if the view Balfour and Parker and I took of it be the right one, it would form a link between that of a frog or newt on the one hand and that of a skate or dog-fish on the other.

This being so, the question of fact becomes of some importance. Dean has certainly had good opportunities for making sure of the point and — my own have been equally good. So far as can be gathered from Dean's statements and from my own abundant material of this period of development, the crux of the matter lies in the mode of preservation. Dean appears to have made no use of osmic acid in his investigations. The only two reagents employed by me were Flemming's fluid and corrosive sublimate.

Eggs preserved in the latter fluid show no signs of the eight furrows described by myself, unless any sublimate remaining in the superficial part of the egg be precipitated by some such reagent as baryta water, but all the eggs of the proper stage (Fig. 3 of Balfour and Parker's memoir) show either four or eight complete furrows reaching to the lower pole. It is so easy to see these furrows in eggs lying in alcohol, or in eggs passed through turpentine and then dried, that I have often demonstrated them to others.

The above brief review emphasizes the differences of opinion among the investigators, and leads one to ask if the cleavage of the egg of *Lepidosteus* shows such wide variations, now cleaving in a holoblastic fashion, and again following the meroblastic form. If these variations do not exist, wherein lies the explanation of these diverse statements? If, on the other hand, they do exist, how shall we interpret so remarkable and exceptional a cleavage, and what new light may it throw upon the problems of gastrulation and embryo formation?

It would seem advisable, before going farther, to point out certain possible sources of misinterpretation.

Although Balfour and Parker state that the segmentation is complete, I think all will agree that this statement should be qualified, since they likewise repeatedly assert that the furrows "nearly meet," and to the latter statement their illustrations conform.

In Beard's still later writings his former observations are reaffirmed. The statement, however, that "*all the eggs of the proper stage show either four or eight complete furrows reaching to the lower pole,*" is not in accord with my findings. Further, so far as I am aware, no other vertebrate ovum exhibits such a marked regularity of cleavage.

The observations on the early stages by Dean are more detailed than those of previous writers, but unfortunately the descriptions and illustrations are made from material fixed in alcoholic picro-sulphuric. The figures given by Dean in his first plate recall the appearances which I have frequently observed in material subjected to the same treatment, but to consider these gross artifacts as normal appearances is a most serious mistake. Dean apparently later realized the unreliability of these surface views, since he states in his paper on *Amia* (p. 414) that he had an "opportunity to observe the living material, and to prepare the figures of those stages especially which in surface view (as my studies of *Acipenser* and *Lepidosteus* had taught) could not well be examined in the fixed material."

As stated in my preliminary note, I firmly believe that, had Dean made his drawings from the living egg and confirmed their correctness by the study of material fixed by a number of reagents, his results would have been in complete agreement with mine. This conviction is supported by the appearance of the marginal grooves shown in Dean's Figs. 8, 11 (Plate I), and further strengthened by a suggestion in a footnote (p. 18), which reads as follows: "The writer has found no segmentation stages in which the furrows extend much lower than the equatorial region of the egg. He does not, accordingly, confirm the note and figures of Balfour, and is inclined to believe that the total segmentation of *Lepidosteus* occurs only as a variation."

It would thus appear that the differences of opinion expressed by Balfour and Parker, Dean, and myself might possibly be explained, but to reconcile these with the views of Beard is impossible. The only conclusion is that either the observations, somewhere, embody most extraordinary errors, or that the cleavage of the egg of *Lepidosteus* is of heterogeneous character—a discovery which would be of no little theoretical import.

A comparative study of the cleavage among the Ganoids reveals in this small group of fishes a most interesting series of transitional forms. Beginning with the holoblastic egg of *Acipenser* which closely resembles the Amphibian egg, we pass to the modified holoblastic as exhibited by the egg of *Amia*, thence to the egg of *Lepidosteus* with its meroblastic tendencies, and from this the typical meroblastic condition of the Teleost is readily derived.

It is a curious and striking fact that in these three Ganoids the first three cleavages are vertical, there being an entire absence of the usual horizontal division occurring in the third cleavage of Amphibia. It is likewise of interest to note that in the egg of *Acipenser*, which approaches most nearly the holoblastic type, we find the greatest percentage of variations in the first, second, and third cleavages; some of these are so marked that one or more of the grooves which form the third cleavage may pass in a horizontal plane. In the modified holoblastic egg of *Amia* the variations are less frequent; rarely, if ever, does a groove of this cleavage pass in a horizontal plane. Variations in the positions of the vertical furrows are numerous; they may pass through the poles forming true meridionals or pass far to one side



of the pole giving rise to a bilateral type (Whitman and Eycleshymer). In *Lepidosteus*, which approaches most closely the meroblastic condition, the variations in the third set are less frequent than in *Amia*. In nearly all cases the grooves pass parallel to those of the first or second, being rarely meridional. In the fourth cleavage there are also interesting transitions, passing from the horizontal of *Acipenser ruthenus* (Salensky) through the modified horizontal or circular of *Amia* (Whitman and Eycleshymer) to the vertical of *Lepidosteus*.

The detailed study of cleavage in certain invertebrate groups, notably the Annelids and Molluscs, has led some writers to regard similarities in cell-lineage as prophetic of relationship. E. B. Wilson well expresses this conception as follows: "When the comparative study of cell-lineage has been carried further, the study of cleavage stages may prove as valuable a means for the investigation of homologies and of animal relationships as that of the embryonic and larval stages." Impressed by the striking homologies in the cleavage of the above forms, I was led at first to search among the vertebrates for similar means of determining relationships. Thus far, however, the facts do not justify the assumption that similarities in the cleavage of vertebrate ova are indicative of relationship.

It is therefore obvious that I cannot agree with Dean that "similarity in the mode of the first four cleavages" is evidence of kinship between *Lepidosteus* and the Teleost. Indeed, the danger of basing speculations regarding relationships upon similarities of cleavage are only made more apparent by my studies. As another illustration I find that Dean assumes from the study of the structure of recent and fossil Amioids that *Amia* is the "most Teleostean Ganoid." A study of its early development led to the belief that the egg is meroblastic, and this, regarded as an established fact, is taken by the author as confirming its close relationship to the Teleost. As a matter of fact, recent researches show the egg to be holoblastic; consequently the claim that it is the "most Teleostean Ganoid" receives no support from early ontogeny.

A comparison of the rate of development in *Lepidosteus* as recorded by the several investigators reveals most striking differences. A tabulation of the times at which development reaches well-marked stages is given below:

Stage of Development	Agassiz	Balfour and Parker	Beard	Falleborn	Dean	Eycleshymer
Late cleavage, cell cap, 100-200 cells.....	.....	48 hrs.+	5-6 hours	.....	25 hours	5-6 hours
Gastrulation complete, blastopore closes.....	.....	.....	24 hours	.....	46 hours	28 hours
Optic vesicles distinguishable.....	.....	60 hours	.....	.....	80 hours	45 hours
Embryo hatches.....	8 days	8 days	7 & 9 days	3 days+	8-9 days	6 days
Pectoral fins appear.....	10 days	10 days	.....	.....	.....	7½ days
Pelvic fins appear.....	19 days	14 days	.....	.....	.....	13 days
Yolk-sac absorbed.....	26 days	26 days	.....	.....	41+	22 days

Balfour and Parker found that on the third day (tabulated as 48 hours+) after impregnation the animal pole is completely divided into small segments, which form a disc similar to the blastoderm of meroblastic ova. Beard states: "The segmentation

is very rapid and a cap of small cells is formed in the course of 5 or 6 hours." According to the data given by Dean (p. 66, Fig. 12, and Plate I, Fig. 12) this stage of development is not attained in less than twenty-five hours. I find that it is reached in five or six hours as recorded by Beard.

The closure of the blastopore, which marks the end of gastrulation, takes place, according to Beard's and my own observations, in about twenty-six to twenty-eight hours; while Dean finds that nearly twice this length of time is necessary for the egg to reach the same stage. Again, as pointed out in a preceding paragraph, a comparison of ages at the time of hatching shows variations, ranging from three days to nine days.

The above considerations lead us to infer that environmental (thermic?) conditions may so modify the rate of growth that a given degree of differentiation may be attained in a fraction of the usual time without inducing pathological changes.

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## EXPLANATION OF PLATES

## PLATE XVII

FIGS. 1-8, 11-15, 19, 20 are drawn from living eggs; the remainder are drawn from eggs fixed and hardened in 10 per cent formaline. The numerals affixed to the cleavage grooves indicate the time at which the grooves reached the points indicated by the dotted lines.

FIG. 1.—Profile view of the unsegmented egg immediately after fertilization, showing natural colors of egg.  $\times 10$ .

FIG. 2.—Profile view of an egg one hour and forty-eight minutes after fertilization, showing the position and extent of the first vertical groove.  $\times 15$ .

FIG. 3.—Profile view of the same egg two hours and thirty minutes after fertilization. The first vertical has extended slightly beyond the margin of the calotte, while the second has barely reached the margin.  $\times 15$ .

FIG. 4.—Profile view of the same egg three hours after fertilization, showing the position and extent of the first three sets of verticals.  $\times 15$ .

FIG. 5.—Profile view of the same egg three hours and thirty-five minutes after fertilization, showing the times at which the grooves of the fourth cleavage appeared and their position with reference to the other grooves.  $\times 15$ .

FIG. 6.—Profile view of the same egg four hours and ten minutes after fertilization, showing the division of the eight central cells, also the addition of more meridionals.  $\times 15$ .

FIG. 7.—Profile view of another egg about five hours after fertilization.  $\times 15$ .

FIG. 8.—Profile view of egg five hours and thirty minutes after fertilization.  $\times 15$ .

FIG. 9.—Profile view of egg about six hours after fertilization.  $\times 15$ .

FIG. 10.—Profile view of egg in late blastula, about seven hours after fertilization, showing extent of cleavage just before gastrulation.  $\times 15$ .

FIGS. 11-15 represent a series of successive cleavages as viewed from the upper pole.  $\times 15$ .

FIGS. 16-18 represent succeeding stages as observed in the preserved material.

FIGS. 19, 20 show some of the more usual conditions observed in the formation of the fourth-cleavage grooves.  $\times 15$ .

## PLATE XVIII

FIGS. 21-24 are diagrams of cleavage phases showing the lines of section of these respective stages.

FIG. 25 is a horizontal section at the level of the nuclei of the four-cell stage, showing the planes of the elongated nuclei preparatory to the third cleavage.  $\times ca. 8$ .

FIGS. 26-28 represent a series of horizontal sections of the blastodisc of an egg in which the grooves are well marked at the level of the nuclei, but have not yet appeared on the surface. An intercellular space is represented which is the beginning of the segmentation cavity.  $\times ca. 9$ .

FIG. 29 is a diagram of an egg in the fourth cleavage, taken in a horizontal plane, at the level of the dividing nuclei, and showing the plane in which the nuclei are elongating for the ensuing cleavage.  $\times ca. 10$ .

FIG. 30 is a vertical section of a mature ovarian egg, showing the surrounding membranes, the micropylar orifice, the germinal vesicle, and the peculiar relation of the finely granular germinal portion to the coarsely granular nutritive portion. The yolk has not been completely filled in.  $\times 18$ .

FIG. 31 represents a horizontal section of a similar stage, somewhat below the level of the equator, and, like the preceding, shows the relation of the finely and coarsely granular portions.  $\times 18$ .

FIG. 32 represents a section of an egg in first cleavage, taken along the dotted line which passes through the center of the egg in Fig. 21.  $\times 18$ .

FIG. 33 is a section of the same egg, but taken nearer the margin of the blastodisc, as indicated by the second dotted line of Fig. 21.  $\times 18$ .

FIG. 34 represents a vertical section of an egg in third cleavage, the section being taken along the dotted line in Fig. 23.  $\times 18$ .

FIG. 35 represents a section of an egg in fourth cleavage along the dotted line in Fig. 29. The section, instead of being vertical, passes obliquely. It will be noticed that the plane of nuclear elongation is that shown in Fig. 29.  $\times 18$ .

FIG. 36 is a section of an egg in the same stage, but passes at right angles to the preceding. The horizontal division of the central cells is shown.  $\times 18$ .

FIGS. 37, 38 represent sections of an egg in the sixth cleavage. The former is taken through the margin of the blastodisc, while the latter passes through the center.  $\times 18$ .

FIG. 39 represents a vertical section of an egg in about the seventh cleavage. The thickness of cell cap, the segmentation cavity, the budding off of cells from the periblast, etc., are indicated.  $\times 18$ .

FIG. 40 represents a transverse section of an egg in the same stage as the preceding. The section is taken midway between the upper pole and the equator, and shows the depth of the cleavage grooves.  $\times 18$ .

FIG. 41 represents a vertical section of an egg in the stage shown in Figs. 9 and 18. Few noteworthy changes have occurred beyond the increased thickness of the cell cap.  $\times 18$ .

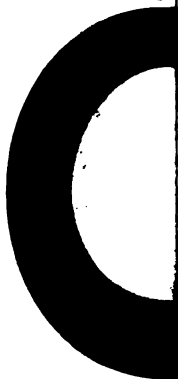
FIG. 42 represents a section of an egg in the stage shown in Fig. 10. The apparent discontinuity of the conical portion of the periblast is due to the obliquity of the section. The stage is that of late blastula. The body of the cell cap is made up of small spherical cells. The outermost layer is differentiated into the superficial epiblast, while the lower layer consists of yolk-laden cells loosely scattered throughout the segmentation cavity.  $\times 18$ .

FIG. 43 is a diagram of an egg in the same stage as 42, but taken in a horizontal plane at the level of the equator, showing the depth of the cleavage grooves at this time.  $\times 18$ .

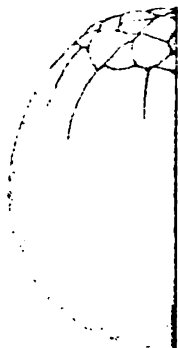


DECENNIAL

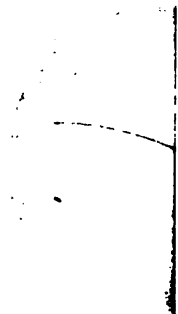
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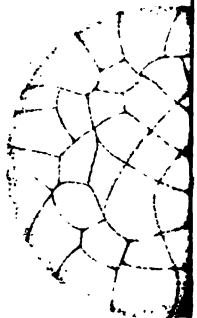
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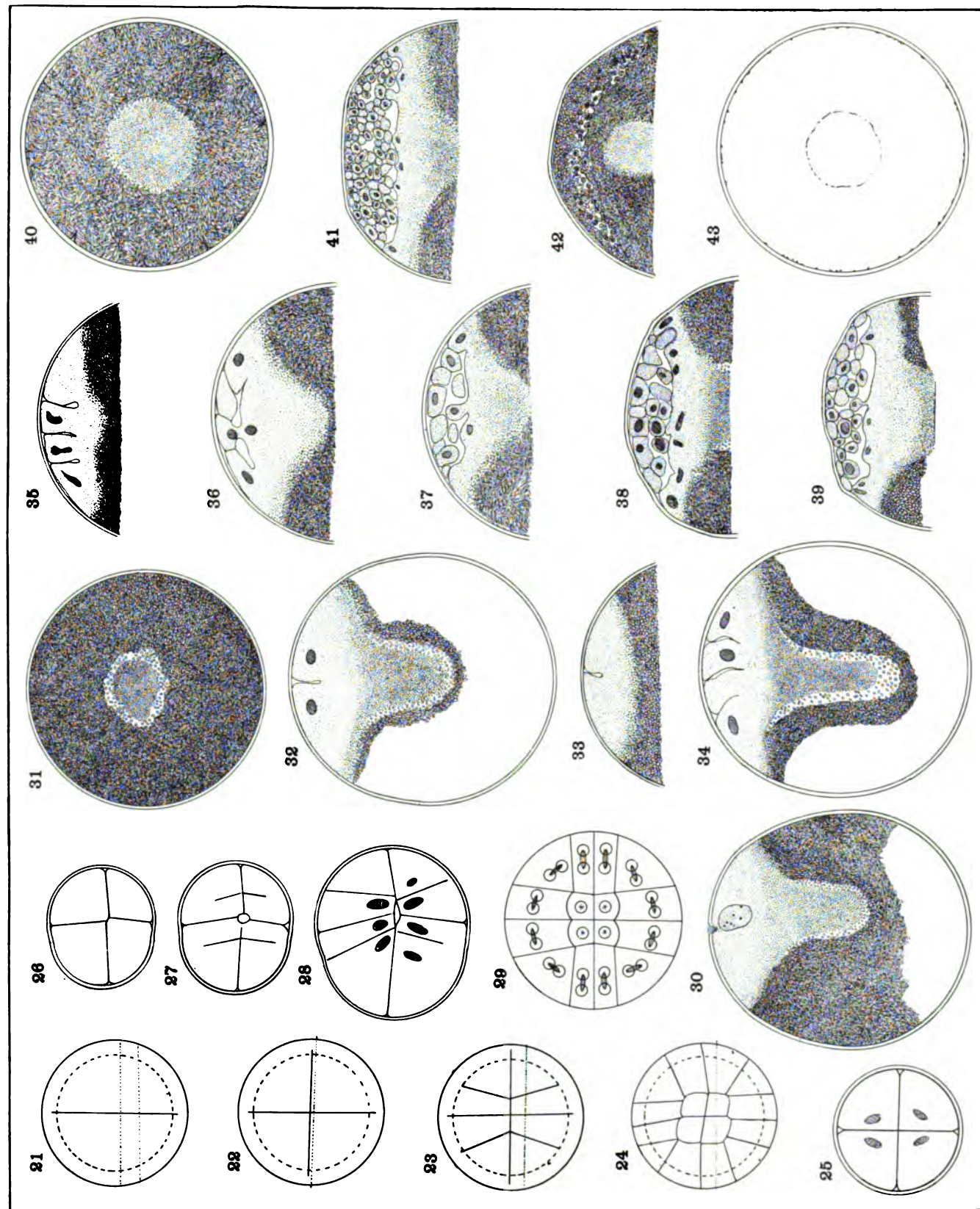
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# **THE STRUCTURE OF THE GLANDS OF BRUNNER**



# THE STRUCTURE OF THE GLANDS OF BRUNNER

ROBERT RUSSELL BENSLEY

## I. INTRODUCTION

THE appearance recently of the papers of Castellant (1898) and Hock (1899), dealing with the structure of the glands of Brunner, is a sign of renewed interest in a group of glands which has baffled both the physiologist and the anatomist. The efforts of the former have been largely directed toward investigating the presence or absence of digestive ferments in the succus entericus or in artificial extracts of the gland. Their investigations, undertaken with this object in view, have not yielded uniform results, although the recent studies of Glassner (1902) confirm the observations of Grützner (1872) that the glands of Brunner contain a proteolytic ferment.

Wepfer (1679), who discovered the glands in 1679, described their arrangement in the duodenum, and observed that when macerated in water they liberated an abundant amount of mucus. Eight years later the glands were described more fully by Brunner, who regarded them as a sort of secondary pancreas. The incorrectness of this view was shown by Claude Bernard (1856) and Middeldorpf (1846), both of whom pointed out that the secretion of the glands of Brunner differed from that of the pancreas.

Budge and Krolow (1870) found that the extract of the glands of Brunner would transform starch into sugar, would dissolve fibrin in acid solution, but would not act on coagulated albumen nor on fats.

Grützner (1872), following up his observations on the proteolytic ferments of the pyloric glands, which he, in common with Heidenhain (1870) and Ebstein (1870), regarded as pepsin-forming glands composed of chief cells like those of the fundus glands, found that he could obtain by extraction of the glands of Brunner with 0.1 per cent. hydrochloric acid a solution which would rapidly digest fibrin in acid solutions.

Similar positive results as to the existence of a proteolytic ferment were obtained by Gachet and Pachon (1898), who introduced cylinders of coagulated albumen into the isolated duodenum after tying the pancreatic duct.

Recently Glassner (1902) has extended to the study of the ferments of the glands of Brunner the methods which he had already employed with success to separate the various ferments of the gastric mucous membrane. The extracts which he obtained, after taking all possible precautions to exclude the glands of Lieberkühn from the material extracted, and to destroy adherent pepsin and trypsin, were inactive with respect to starch, cane sugar, and fats. On the other hand, they digested fibrin, serum albumen, and coagulated egg albumen in solutions containing 0.2–0.3 per cent. of hydrochloric acid. Moreover, some proteolytic activity was still displayed when

the solutions were rendered neutral or slightly alkaline. The specific ferment upon which this proteolytic action depended, he identified with the pseudopepsin which he had previously extracted from the pyloric mucous membrane. It differed from pepsin in that it was not destroyed, nor its activity removed, by weak solutions of sodium carbonate, and that its action quickly led to the formation of tryptophan. From trypsin it was distinguished by its activity in acid solutions.

In view of these experiments of Glässner, Grützner, and others, it seems certain that the cells of the glands of Brunner contain a proteolytic enzyme. As yet, however, it is not known whether this is a tissue enzyme concerned in some of the various metabolic processes of the cell itself or a secreted product of the cell designed to assist in the intestine, in the transformation of the proteids of the food.

Anatomically the main points of interest, as far as the glands of Brunner are concerned, have been the form and distribution of the glands, the question as to whether they are mucous or serous glands, the changes they exhibit in different stages of physiological activity, their relationship to the pyloric glands, and their phylogeny.

The question of the form of the glands has been adequately treated by Schwalbe (1872), whose conclusions that the glands of Brunner are composed of ramifying tubules into which acini open have been confirmed with some additional details, by the studies of Maziarski (1902) and Peiser (1903), who employed the reconstruction method of Born.

Concerning the mucous or serous nature of the glands, however, there is not the same unanimity of opinion. By many authors, including Schwalbe (1872), Heidenhain (1872), Bentkowski (1876), and Piersol (1894), they have been compared with the pyloric glands which these authors regarded as similar to the chief cells of the fundus glands. Claude Bernard (1856), Sappey (1876), Renaut (1879), and Berdal (1894) regarded them as mucous glands. Renaut (1879), basing his conclusions on the study of the glands of Brunner of man, regarded them as structures differentiated for the secretion of a peculiar mucus. He compared them with the pure mucous glands of the oesophagus and bronchi, which, according to him, have the same fundamental structure as the glands of Brunner.

The same view, in a somewhat modified form, was taken by Kuczynski (1890), who studied in a number of representative mammals the staining reactions of the glandular cells by means of certain synthetic dyes, in particular victoria blue, azoblue, aniline blue, and thionine. These he found to stain the cells of the glands of Brunner of different mammals with different degrees of facility. Some cells, for example those of the pig, were refractory to all attempts to stain them. He concluded that in the latter animal the cells contained no mucin; that in others, for example the rabbit and horse, where the staining was feeble, the amount of mucin was small; and again that in the guinea pig and ox, where the cells stained strongly, they contained a large amount of mucin.

Schaffer (1891) obtained a slight violet coloration of the cells of the glands of

Brunner of man with hæmatoxylin; and concluded that the mucus which they secreted differed materially from that formed by goblet cells and by the salivary glands.

Castellant (1898) concluded that the glands of Brunner could not be regarded as mucous glands, but should rather be compared with the pyloric peptic glands. He added, however, that the differences, considerable in the rat, slight in the dog, between the glands of Brunner and the pyloric glands, led him to believe that their secretion was not identical, but that the glands of Brunner secreted a special digestive liquid.

A similar view of the nature of the Brunner's glands is taken by von Ebner (1899), who bases his conclusions as to their non-mucous nature on the failure to stain them with Mayer's mucicarmine and muchæmatein.

The lack of unanimity of opinion as to the nature of the glands of Brunner is to be traced to a number of causes. Of the first importance, in this connection, is the resemblance of the glands of Brunner to the pyloric glands of the stomach which Heidenhain (1870, 1878) and his pupils had shown, apparently conclusively, on physiological grounds, to be pepsin-forming serous glands. This conclusion, as far as the pyloric glands are concerned, has been shown to be erroneous by the writer (1896, 1898) on histological and microchemical grounds, and for chemical reasons by Glässner (1902), who has confirmed the conclusion of the writer that the glands of the pyloric region do not contain pepsin-zymogen (propepsin). A second cause which has contributed to this confusion of results is the lack of precision in our morphological criteria for distinguishing between mucous and serous cells. In the absence of specific knowledge of the chemistry of the secretion of a gland, a mucous gland in which the nucleus was spherical and the cytoplasm abundant would invariably, according to the older ideas, be interpreted as a serous gland.

The classification of glands into mucous glands and serous glands is at the best a mere makeshift. When we have decided that a gland is a serous gland, we may still know absolutely nothing about the nature of its secretion, beyond the fact that it does not contain a mucin. There are, however, a number of serous glands the secretion of which it is possible to collect and examine chemically, and which are known to be largely engaged in the secretion of digestive enzymes. To this category belong certain of the serous salivary glands, the pancreas, and the chief cells of the gastric glands. In recent years these zymogenic glands have been the subject of numerous investigations, as a result of which we now have a tolerably exact knowledge of their structure in the different phases of physiological activity. The serous glands have been investigated by Solger (1894, 1896, 1898), Erik Müller (1895), Zimmermann (1898), Garnier (1900), and others; the pancreas, by Eberth and Müller (1892), Mouret (1895), Macallum (1891, 1895, 1898), and Matthews (1899); the gastric glands, by Bensley, (1896, 1898, 1902), Zimmermann (1898), Theohari (1899), and Cade (1901).

These researches show that serous glands which are known to be zymogenic in function, whatever their source, have certain features in common, due to the presence

in the cell of the several substances antecedent to the secretion. These are, briefly, the *zymogen* granules which occupy the portion of the cell nearest the lumen, and which are easily visible in the fresh cell, and the *prozymogen*, a nucleoproteid substance probably of nuclear origin, remarkable for its staining power, located in the base of the cell. The latter substance is often unequally distributed in the basal cytoplasm, giving rise to the appearance of radial striation, to which we owe the name "basal filaments," first employed for the prozymogen of the glandula submaxillaris of man by Solger (1896).

Both of the substances mentioned above as characteristic constituents of the zymogenic cell give microchemical reactions which enable one to distinguish them with some confidence from the substances antecedent to secretion in a mucous cell. The fact that the prozymogen is a nucleoproteid enables one to employ the microchemical tests for iron and phosphorus for its identification, and no structure in any cell should be likened to the basal filaments of the serous zymogenic cell unless it does give a positive result with these tests. Furthermore, the granules of zymogen may be positively distinguished from granules of mucigen by the fact that after careful extraction with alcohol and ether in a Soxhlet apparatus to remove the lecithins, they give a strong reaction by Macallum's method, showing the presence in them of organic phosphorus as an elementary constituent.

Mucous cells, on the other hand, do not contain any basal filaments, and the feeble reaction for organic iron which they give indicates that they contain a relatively small amount of diffused prozymogen. As pointed out above, the secretion granules of mucous cells do not give any reaction for organic phosphorus.

In addition to the above characters which we can apply in distinguishing serous from mucous cells, we are able, owing to the researches of Paul Mayer (1896) on the methods of staining mucus, to obtain, with much greater certainty than before, a positive staining reaction for this substance in cells by employing the special solutions of hæmatein and carmine devised by him.

Up to the present, there has been no attempt to apply these methods to the study of the glands of Brunner. We must, therefore, compare these glands as regards the structure, staining and microchemical reactions, and the changes exhibited by them in different phases of functional activity, not only with the nearly adjacent gastric and intestinal glands, but also with the many glands from other sources which have been the subject of exact investigation.

A point on which most writers agree is the great similarity between the glands of Brunner and the pyloric glands of the stomach, although recent researches of Kuczynski (1890), Castellani (1898), and others do not confirm the conclusions of Bentskowski (1876) and Schiefferdecker (1884) that the two sorts of glands are identical.

The question of the relationship of the glands of Brunner to the pyloric glands is mainly interesting from the standpoint of the phylogenesis of the former. Although

they occur for the first time in mammals, the fact that they have been found without exception in all mammals belonging to the three main subclasses in which they have been sought, indicates that they must have appeared very early in the history of the Mammalia. The fact that the pyloric glands and the Brunner's glands have been subject throughout this long phylogenetic history to conditions which are not identical would alone be sufficient to explain slight differences in the nature of their cells, even if, as some suspect, the two sets of glands have had a common origin. Furthermore, in many groups of animals, owing to a change of food habits, the stomach has undergone important secondary adaptive modifications, resulting in the suppression of extensive areas of gastric glands, the replacement of the cylindrical epithelium by a stratified squamous epithelium, and the modification in type of the remaining glands. It is reasonable to expect that, under these extraordinary conditions, the pyloric glands would be modified, and the differences between them and Brunner's glands intensified. Clearly, it would be unfair to assume that, because the pyloric-gland cells differ from those of Brunner's glands in an animal with such a specialized stomach, they are primitively different. In other words, in discussing the question of the difference or similarity of the two sets of glands, both the long phylogenetic history of the glands and the relative visceral specialization of the animal under discussion must be given due weight.

The present memoir embodies the results of an investigation undertaken with a view of applying to the solution of the problems presented by the glands of Brunner the microchemical and staining methods employed by the writer in the study of the glandular elements of the stomach, and the more recent conception of the structure of these and other cells. With this end in view, the glands of Brunner of one marsupial and of nineteen placental species representing six orders of mammals have been submitted to a careful anatomical and histological examination. The results from a phylogenetic standpoint are somewhat disappointing, but it is hoped that they will bring us nearer to a proper conception of the morphology of the glands in question.

The material for study included the pyloric glands and glands of Brunner of the opossum, dog, cat, mink (*Lutreola*), raccoon (*Procyon*), hedgehog (*Erinaceus*), porcupine (*Erethizon*), guinea pig, ground hog (*Arctomys monax*), squirrel, rabbit, dormouse (*Muscardinus avellanarius*), muskrat (*Fiber zibethicus*), mouse, rat, deer mouse (*Peromyscus*), sheep, pig, and man.

The glands of Brunner of the opossum have been selected for the preliminary description, because the shape and structure of the stomach in this form correspond so closely to those which we have reason to believe were primitive in mammals that we may expect to find primitive conditions in the glands, and because the peculiar characteristics of the glands of Brunner in the opossum furnish, it is thought, the strongest argument which could be brought forward in favor of the theory that they are produced directly from the pyloric glands.



II. THE GLANDS OF BRUNNER OF *DIDELPHYS VIRGINIANA*

The glands of Brunner in the opossum form a narrow ring around the pyloric orifice of the stomach. In a preparation of the stomach and duodenum, cut open along the line of attachment of the hepatogastric and hepatoduodenal ligaments, one may see in the mucous membrane at the gastro-duodenal junction with a low magnification, a number of minute funnel-shaped depressions, forming two or three irregular rows more or less parallel with the line of junction of the stomach and intestine, and at distances of two to three millimeters apart. On examination in sections these prove to be the openings of tubular depressions of the mucous membrane into which the glands of Brunner open. These depressions are not, however, in the strict sense, the ducts of the glands, but rather evaginations of the mucous membrane as a whole, because, in the inner portions of them, villi project from the wall into the cavity, and small intestinal glands occur, beneath which a continuation of the longitudinal fibers of the lamina muscularis mucosæ may be seen. These intestinal elements may be traced to a short distance below the level of the lamina muscularis mucosæ of the stomach and intestine, where the depression opens into a cavity of considerable size, around which the lobules of the glands of Brunner are clustered, and into which the ducts of the latter open. If the stomach and intestine, and after ligation of the latter some inches below the pylorus, be distended with the fixing fluid, under sufficient pressure to overcome the resistance of the pyloric sphincter and to dilate the opening to a width of about 1.5 cm., one sees at the gastro-duodenal junction, instead of the depression referred to above, a number of rounded patches which are smooth and devoid of villi. In sections of such a preparation, the appearance represented in Plate XIX, Fig. 1 is observed; the lobules of the glands of Brunner are clustered around a place where the ordinary elements of the intestinal mucous membrane are lacking and only an epithelium is to be seen. Through large openings in this epithelium empty the ducts of the glands of Brunner. The first row of these defects in the intestinal mucous membrane occurs at or near the gastro-duodenal junction, that is to say, the first row of patches into which the glands of Brunner open may be continuous with the mucous membrane of the stomach, or may be separated from the latter by a minute interval, about 0.1 mm. in width, in which villi, intestinal glands, etc., may be found. In the former case, the glands of Brunner have the appearance of being a continuation of the pyloric glands; in the latter, they appear to be entirely separated from them.

The extent of the glands of Brunner in a longitudinal direction is about 6.7 mm., beginning about 1.2 mm. above the point where the gastric and duodenal epithelium meet, and extending a distance of 5 mm. to 5.5 mm. into the tela submucosa of the duodenum. They form a series of angular lobules, some entirely separate, others aggregated into larger or smaller lobes.

The glands are of a branched acinotubular type. The smaller lobules have as a rule a single tubular duct which opens on the surface of one of the depressions above

described. This duct penetrates to the center of the lobule giving off radial tubular branches which subdivide repeatedly until an exceedingly complex structure is produced. All tubules below the main duct give off at frequent intervals short tubules which are regarded by Mażiarski (1902) as acini. The cells lining all these branches of the main duct, and indeed those of a large portion of the duct itself, are of precisely the same character.

The tubules are surrounded and supported by the collagenic connective tissue of the tela submucosa, in which may be seen near the epithelium of the defect small strands of smooth muscle fiber which represent the remains of the lamina muscularis mucosæ. In the tissue between the glands numerous mast cells may be seen, some lying free in the connective tissue, others closely applied to the outer surface of the glandular epithelial cells.

The defect of the tunica mucosa, into which the ducts of the glands open, is covered by a single layer of epithelial cells. At the edge of the patch these consist of the usual epithelial elements of the intestine, namely goblet cells, cylindrical epithelial cells with basal cuticula, and granule cells of Paneth. Epithelium of this character, however, extends over a very small portion of the extreme margin of the patch and is broken by only a few ducts. The greater portion of the area is covered by cylindrical epithelial cells of the mucigenous type. The shape and dimensions of these surface epithelial cells vary within wide limits, according to the tension and shape, that is, convexity or concavity, of the surface upon which they rest. On relaxed portions of the surface, they are very high and narrow, 17–27  $\mu$  in height by 5–7  $\mu$  in width. If, however, the tissue is fixed under tension, they appear short and wide and the position and shape of the nucleus are similarly modified.

At first sight the resemblance of the epithelium to that of the stomach is remarkable, but on close examination it is found that this resemblance does not amount to identity, although the differences are not of great importance. As in the gastric epithelium, the distal portion of the cell is occupied by a mass of secretion forming a distinct theca. The proximal portion of the cell is occupied by finely reticular cytoplasm and contains an oval nucleus. The latter may be slightly flattened where it comes into contact with the mass of secretion. The differences between these cells and those of the gastric epithelium are particularly emphasized in specimens stained in iron alum hæmatoxylin, in muchæmatin, or in mucicarmine. In the former stain the theca of the gastric epithelial cell appears of a slightly grayish color and homogeneous. The masses of mucin (granules?) which fill the theca are so closely aggregated that there is but little cytoplasm left in the free ends of the cell to retain the stain. At the base of the theca only, can be made out a delicate network, which extends from the cytoplasm between the theca and the nucleus for a short distance into the mass of secretion. In the epithelial cells of the Brunnerian area, on the other hand, the theca is subdivided by delicate strands of cytoplasm forming a network in the meshes of which the granules of mucin are lodged. Moreover, the theca is subdivided into proximal and

distal portions by incomplete bands of cytoplasm, stretching across it, parallel with the free border of the cell. The meaning of these facts will be discussed later. In the meantime it is merely intended to point out that the theca of the epithelial cells under discussion contains a larger proportion of cytoplasmic elements, as distinct from stored-up secretion, than that of the epithelium of the stomach. A further point of difference is to be seen in the size of the theca, which presents a remarkable uniformity in the gastric epithelium, but is very variable in the epithelium of the defects.

Toward the bottom of the gastric foveolæ, however, cells are found which agree very closely in structure with the cells of the defects, and these, as has been frequently pointed out, by Bizzozero (1893) and others, are connected with the cells of the surface by a gradual transition. A similar transition may be seen at the gastro-duodenal junction, where the epithelium of one of the defects happens to be continuous with the gastric epithelium. *We may therefore conclude that the epithelium of the defects is gastric epithelium not so highly differentiated as that of the surface of the stomach.*

The ducts which open on the defects are lined for a greater or less portion of their course by epithelium of the type described above, except that the cells are as a rule shorter and wider than on the free surface. At variable distances from the opening of the duct, there is transition, sometimes abrupt, sometimes gradual, to the glandular epithelium which lines all the numerous side and terminal branches of the gland.

The two animals from which the material was obtained exhibited different physiological phases of the gland; in one the cells were completely filled with secretion, in the other only partly so. A transverse section of two tubules from the latter animal is represented in Plate XX, Fig. 2. Each tubule is composed of somewhat rectangular cells, very similar in general characters, but with some differences of detail, surrounding a central cavity. The lumen varies in width from  $4\mu$  to  $16\mu$ , the largest diameter being usually found in the main branches of the duct and in those terminal tubules (acini) which lie at the margin of a lobule. In each glandular cell a number of distinct zones may be made out. Beginning at the outside of the tubule and proceeding toward the lumen, there may be distinguished, first, a narrow zone of cytoplasm, of a delicate reticular structure, stretching across the base of the cell and containing the somewhat irregular nucleus. On the distal side of the nucleus a clear zone with coarse reticular structure may be observed, then a narrow band of finely reticular cytoplasm, and finally a second coarsely reticular clear zone, bordering the lumen. The meshes of the two clear zones obviously contain the stored-up secretion of the cells, which is divided into a proximal and a distal mass by the transverse bridge of cytoplasm. This reciprocal arrangement of the cytoplasm and its product is deserving of some emphasis because of the fact that it occurs with surprising constancy in corresponding phases of secretion in mucous cells from the most varied sources, *e. g.*, salivary, palatine, œsophageal, and tracheal glands, the cardiac and pyloric gland cells of the stomach, and the neck chief cells of the fundus glands. Zimmermann (1898) observed this structure in the cells of the human glands of Brunner, but did not attempt an interpreta-

tion. Kolossow (1898), by means of his osmic-acid reduction method, obtained similar pictures in the salivary glands, and recently Maximow (1901) has demonstrated it by thionin staining in the mucous salivary glands. The writer has repeatedly called attention to this phenomenon in the mucous-secreting cells of the stomach. A character which reappears with such constancy in similar cells from so many different sources must have a very important significance.

The network which is visible in the clear zones of these cells is neither a true cytoplasmic reticulum (spongioplasma) nor an alveolar structure (*Wabenstruktur*), but is probably a derivative of the latter. In sections stained intensely in iron alum hæmatoxylin it is possible to follow the threads of the network by focusing. In such preparations it is seen that, in a great many cases, they are not fibers, as would appear at first sight, but thin laminæ which intersect and join to form the visible network. The spaces of this network are, however, everywhere continuous with one another. Often, however, the proximal mass of secretion near the nucleus exhibits a true alveolar structure, the spaces being rounded cavities containing reserve secretion, separated completely from one another by the continuous cytoplasm of the zone. It is easily conceivable that the latter structure has given rise to the former by the increase in size and partial coalescence of the small secretion spaces. Usually there is a very obvious difference in the amount of residual cytoplasm contained in the proximal and in the distal clear zones respectively, the proximal zone exhibiting smaller spaces and larger cytoplasmic trabeculæ than the distal zone.

The cytoplasm of these cells does not contain basal filaments, but the presence of a small amount of cytoplasmic nucleoproteid is indicated by the feeble but positive reactions for iron obtained by Macallum's methods.

In order to study under the best conditions the reciprocal relations of the cytoplasm and secretion, it was necessary to have an intense stain of the masses of secretion, leaving the cytoplasm unstained. The various synthetic dyes which were tried did not yield very satisfactory results, as, although a positive result was obtained with thionin, toluidin blue, and methyl blue, the stain was not sufficiently selective to permit of accurate definition of the cytoplasm and secretion respectively. By means of P. Mayer's muchæmatein the writer obtained a very intense and satisfactory stain of the secretion by transferring thin sections cut in paraffin, from benzole to absolute alcohol, and then to the stain, but was unsuccessful with similar sections fastened to the slide. It was subsequently found that, by gradually increasing the strength of the solution without altering the relative proportions of its solid constituents, a solution was obtained which could be used for staining sections fastened to the slide, with certainty of speedy and satisfactory results. The procedure is as follows: The stain consists of hæmatein, 1 g., aluminium chloride, 0.5 g., 70 per cent. alcohol, 100 c.c. The hæmatein and chloride are rubbed up together in a mortar, then mixed with the alcohol, the whole being allowed to stand a week to insure perfect ripeness of the solution. During this week the solution deepens in color, and its staining power for mucin

increases daily. The sections fastened to the slide by the water method are treated with benzole and absolute alcohol. The slide is then flooded with the staining solution, placed on the stage of the microscope, and watched until the intense blue color appears in the cells. The sections are then rapidly washed in 95 per cent. alcohol, dehydrated, cleared, and mounted in xylol balsam. Washing in water extracts the stain, but if the sections are first washed in 70 per cent. alcohol, then in lime water, the stain is fixed, and subsequent washing in water affects it but slowly. Similarly effective results may be obtained with mucicarmine, if the strong stock solution of Mayer be employed instead of the diluted solution. Mucicarmine can be depended on only if the solution is freshly prepared; the old solutions do not give satisfactory results. The stain obtained with mucicarmine is very stable, is not affected by washing in water, and may be used for subsequent contrast staining. Very excellent double stains, remarkably rich in detail, are obtained by staining in iron alum hæmatoxylin, followed by mucicarmine.

Muchæmatein, prepared and applied in the way described above, gives an intense blue color to the contents of the goblet cells and of the clear portion of the glandular epithelial cells of Brunner. Old solutions stain the granules of the mast cells and, if the solutions are acid, as Harris has pointed out, the coarser elastic fibers. Cytoplasm remains unstained. Cells stained in this way exhibit the exact reverse of the structure described above, and illustrated in Fig. 2. The clear zones of the cell appear filled with deeply stained secretion, the rest of the cell colorless.

Considerable interest attaches to the mode of aggregation of the secretion in the cell, and in this connection some results have been obtained which afford a simple explanation of the discordant results on the mucous salivary glands. The writer was at first considerably puzzled by the fact that in some of his preparations the secretion appeared in the form of distinct granules, in others in the form of a continuous coarse meshed network. It was speedily found, however, that if water were excluded from the operations of the technique, the granular condition was always obtained, whereas if water were introduced at any stage, the reticular appearance was obtained. For example, sections stained without fastening to the slide, or after cutting in celloidin, by simply transferring from strong alcohol to the stain, then back to alcohol, gave granules; sections passed through water, or fastened to the slide by the water method and heat, gave a network. The obvious inference was that the secretion was stored in the cells in the form of fine granules or droplets which had not been altered chemically by the fixing agents, and which on treatment with water promptly went into complete or partial solution, to be again precipitated by the stain in reticular form. It is a well-known fact that mucins outside the cell precipitate frequently in the form of a coarse network.

We may now return to a description of the cell after staining with muchæmatein in such a way as to preserve the granules of secretion. Such a preparation is illustrated in Plate XX, Fig. 3. In each cell there are seen to be two masses of

granules corresponding to the two clear zones of the cells of the preceding figure. The granules in the distal mass bordering on the lumen are very closely aggregated, very small, and somewhat angular in outline. Those of the proximal mass are less closely packed, often somewhat scattered, smaller and more rounded.

If we now combine the results of the two methods of staining, we may conceive the clear secretion-filled zones of the fixed cell to be composed of two elements, strands, threads, and delicate laminæ of cytoplasm, forming a network, in the meshes of which are minute granules of secretion, the latter separated from one another, sometimes by the threads of cytoplasm, more often by clear spaces. This appearance may be interpreted in one of two ways. In the living cell the granules of secretion must be separated from one another by a continuous substance of some kind. This continuous separating substance may be either the cytoplasm or a third substance of a more watery nature, filling the interstices of the cell between the particles of secretion and the cytoplasm. In the former case the strands of cytoplasm seen in the dead cell would represent merely the contracted precipitates produced in the continuous cytoplasm by the fixing reagents; in the latter case they would represent the actual distribution of the cytoplasm in the living cell. The latter interpretation seems the more probable for a number of reasons. In the first place, it suggests a possible explanation of the capacity of the cell to vary the respective constituents of its secretion in response to specific stimuli, the possibility of which has been clearly demonstrated by the work of Pawlow (1898) and his pupils on the stomach, and by that of Malloisel (1902) on the submaxillary gland. In the second place, one frequently sees granules with minute threads of substance stainable in muchæmatein projecting from their surfaces suggesting that there is an intermediate clear substance (the hyaline substance of Langley) in which portions of the secretion of the cell exist in complete solution. A third reason, perhaps a stronger one than either of the two foregoing, is that one finds the secretion in the form of droplets or granules in the theca of goblet cells from some sources, in which strong staining in iron hæmatoxylin shows the presence of only faint, delicate threads of cytoplasm, or of none at all.

A glandular tubule from a section fastened to the slide by the usual water method and stained in muchæmatein is represented in Plate XXI, Fig. 8. The granules of secretion have disappeared and have given place to the coarse network usually seen in mucous cells. The division of the accumulated secretion into an inner and an outer mass may still be recognized in some of the cells, although the bridge of protoplasm separating these is less obvious in the midst of the deeply stained secretion. This network again is not of an alveolar character, as the laminæ and trabeculæ which form it are perforated and interrupted in such a way that the clear spaces throughout the mass communicate with one another.

The blue-stained network observed in muchæmatein preparations is a precipitation product, and must not be confused with the network visible in the clear zones of cells stained by the iron-hæmatoxylin method. The mucinoid material is doubtless precipi-

tated in part on the cytoplasmic meshes, but not wholly so, because the network obtained in muchæmatein is formed by much larger, coarser meshes than that seen in the iron hæmatoxylin preparation. Moreover, one may completely remove the substance stainable in muchæmatein by treatment of the sections for several hours with a dilute solution of barium hydroxide. Subsequent staining in iron hæmatoxylin shows that the cytoplasmic network in the clear zones of the cell has undergone no change.

We may now consider the changes exhibited by the cell while passing from the intermediate state described in the foregoing paragraph to the fully loaded conditions. As already pointed out, one may find in the same tubule cells in the different secretory conditions, and by comparing cells from different tubules an idea may be obtained of their secretory phases. The majority of the cells in Fig. 2 are in a condition intermediate between the loaded and the discharged states. The completely loaded stage is represented in Plate XX, Fig. 4, drawn from a tubule of a gland of Brunner of a second opossum, stained with iron hæmatoxylin. In this preparation the cell presents a swollen aspect and exhibits throughout a coarse meshwork of cytoplasm inclosing clear secretion spaces. There is no indication of a subdivision of the contained secretion into a proximal and distal mass, the transverse bridge of protoplasm seen in most of the cells of Fig. 2 being here represented only by a slight thickening of some of the cytoplasmic trabeculæ in the middle of the cell. The cytoplasm at the base of the cell, which was of considerable extent in Fig. 2, is here reduced to a minimum, and the nucleus has in many cells taken on the crescentic form so characteristic of mucous cells. The tubules of the gland in this individual were in general larger and the lumina narrower than in those from the first animal. An idea of the way in which this secretion-loaded cell is derived from those of the type illustrated in Fig. 2 may be gained by studying a large number of tubules from the same animal stained in iron hæmatoxylin and in muchæmatein. It has been pointed out that in preparations stained with muchæmatein without coming in contact with water the secretion appears in the form of rounded granules arranged in two more or less distinct groups, and that the granules of the proximal mass are frequently smaller and less crowded than those of the distal mass. The changes in the cell in the act of storing up secretion seem to be going on more actively in this inner mass of granules which increases progressively in density and extent by increase in the size of the granules and by addition of new granules. Correlated with this increase in amount and number of the droplets of secretion is a diminution of the amount of cytoplasm which takes place simultaneously in the basal cytoplasm, in that of the transverse bridge, and in that of the intergranular trabeculæ. The result is that the transverse bridge of cytoplasm presently disappears and the two masses of secretion become continuous. At the same time the amount of secretion in the distal zone is probably increasing, although this is less easily made out. A comparison of the cells in the two extreme conditions stained in iron hæmatoxylin shows that the cytoplasmic network of the distal zone is composed of larger meshes in the fully loaded cell.

A feature of some interest is the relation of the granules of secretion, as seen in muchæmatein preparations, to the nucleus of the cell. Frequently only one of the two masses above described may be seen, namely that which corresponds to the proximal mass, the edge of the cell along the lumen, in this case being cytoplasmic in nature. The obvious interpretation of this fact is that at the last period of activity this cell has thrown out all of its reserve secretion and that, while this has been going on, the cytoplasm has been increasing in amount and new granules have been forming in the proximal segment of the cell between the nucleus and the mass of old secretion. In the majority of cells, however, only a portion of the reserve secretion is so discharged and new granules are deposited alongside of the old ones in the interior of the cell.

The formation of new secretion in mucin-forming cells in close proximity to the nucleus has been observed by Krause (1895) in the cells of the retrolingual gland of *Erinaceus*, and more recently has been demonstrated by Maximow (1901) in the cells of the retrolingual gland of the dog. The meaning of this phenomenon is not clear, for although Carlier (1899) has demonstrated in the large mucous cells of the gastric glands of the newt, morphological changes of the nucleus in the different phases of secretion identical with those exhibited by the pepsin-forming cells, yet microchemical study does not reveal in mucous cells, as it does in many serous cells, the presence in the cytoplasm in large amount of an undoubted product of nuclear activity (prozymogen, ergastoplasm of Cade and Garnier). It is possible that the transformation of the substances received by the cell into mucin is accomplished by the agency of an enzyme formed in the nucleus. A further possibility is the effect of the presence, in relation with the proximal mass of secretion of the canals of Holmgren's trophospongium, although I have not yet been able to demonstrate such canals in the cells of the glands of Brunner.

If one compares the foregoing description of the cells of the glands of Brunner with the account of the cells of the retrolingual gland of the dog, recently published by Maximow (1901), he cannot fail to be struck by the extraordinary resemblance, extending even to the minutest details, between these two kinds of glands. In fact, if we leave out of account the serous tubules and cells of the retrolingual gland, the description would apply equally well to both. In the case of the glands of Brunner, it is not possible to collect the secretion as it flows from the gland and examine it chemically, but there are many reasons for supposing it to consist, like that of the retrolingual gland, largely of mucin. These reasons are briefly: (1) the strong resemblance in structure and physiological phases to cells known to be engaged in the secretion of mucin, *e. g.*, glandula sublingualis of the dog; (2) the stain, obtained in strong muchæmatein and mucicarmine, in which the protoplasm of the cylindrical, intestinal epithelium cells and the granules of the cells of Paneth remain colorless; the feeble metachromatic stain occasionally obtained in thionin; and (3) the solubility in weak alkaline solutions. The latter fact I have established by using muchæmatein as an indicator. I found that if sections attached to the slide were treated with a 5



per cent. solution of potassium carbonate, or a saturated solution of barium hydroxide, the substance in the cells of the glands of Brunner slowly dissolved. The various stages of this slow solution could be determined by treating sections from time to time with strong muchæmatein. The procedure is as follows: A number of sections fastened to slides by the water method are freed from paraffin, passed through alcohol to water, placed in a Coplin jar containing a quantity of the solution to be tested and the whole placed in an incubator at 37° C. From time to time slides are removed from the solution, carefully washed in water, and in dilute acetic acid (0.1 per cent.) if the solution used was alkaline, again washed in water, transferred to alcohol, stained in muchæmatein, cleared and mounted in balsam. In this way the steps of the reaction, if any, could be accurately followed. I found that the contents of the glands of Brunner placed in a solution of barium hydroxide saturated at room temperature disappeared in two hours; that of the pyloric gland cells, of the gastric epithelial cells, and of the goblet cells required ten to twenty-four hours for complete solution. The granules of the Paneth cells were unaffected. The mucin from all the above cells was unaffected by treatment with 5 per cent. solution of hydrochloric acid for twenty-four hours at 37° C. or by peptic digestion for the same length of time. The difference in the solubility of the mucin from the several sources—Brunner's cells, gastric epithelium, goblet cells, etc.—may be due to the unequal action of the fixing fluid (alcohol bichromate sublimate), but, on the other hand, may indicate a chemical difference in the nature of the mucins from these several sources.

The cells of the pyloric glands in the opossum resemble the cells of the glands of Brunner very closely. The differences which may be observed are of secondary importance. The tubules of the pyloric glands and the cells composing them are distinctly smaller than those of the glands of Brunner. The internal structure of the cells is, however, almost identical. The most obvious differences between the pyloric glands and the glands of Brunner consist in the great number of cells of Stöhr visible in the former and in the greater ease with which the contained secretion may be stained with muchæmatein. The mucin of the pyloric glands may be stained by a weaker solution of muchæmatein, in which the glands of Brunner remain colorless.

### III. THE GLANDS OF BRUNNER OF THE CARNIVORA

Next to the opossum in order of simplicity, as regards the structure of the glands of Brunner and their relation to the pyloric glands, come the genera of the placental orders, Carnivora and Insectivora. The structure and extent of the glands of Brunner in the cat and dog have been the subjects of numerous researches and are well known. The facts as they present themselves in these animals are the foundations for most of our knowledge, both physiological and histological, concerning the glands of Brunner.

From the standpoint of specialization, the glands of Brunner of the cat and dog present an advance on the condition found in the opossum, inasmuch as the ducts,

instead of opening in groups on a special area of the mucous membrane from which glands of Lieberkühn and villi are absent, penetrate between the former, traverse the whole thickness of the mucous membrane, and open independently, on the surface, between the bases of the intestinal villi. The epithelium at the mouth of these ducts presents, as Schiefferdecker (1884) has pointed out, a quite remarkable resemblance to gastric epithelium, the same differences, however, being recognizable, as have been already described for the epithelium of the ducts of the Brunnerian area of the opossum.

The resemblance of the cells of the glands of Brunner of these animals to those of the pyloric glands is a very close one—so close, indeed, that one is justified in declaring that they are identical. In the glands of Brunner of the cat the subdivision of the secretion into two masses is very obvious; in the dog, in the resting condition, the cell is so filled with secretion that such a subdivision cannot be made out, but if the cells be exhausted by stimulation with pilocarpine or by feeding, it is seen that new secretion is deposited in the neighborhood of the nucleus and forms a proximal mass. The essentially similar changes in the pyloric-gland cells under experimental conditions of secretion have already been described by the writer (1898). The differences between the pyloric and Brunner's glands of these animals consist in the greater size of the constituent tubules, to a large extent due to the expansion of the lumen, and in the absence of the granular cells of Stöhr from the glands of Brunner. Another difference which is particularly obvious in the cat is the greater variability of the cells of Brunner's glands in respect to the amount of mucin they contain.

In both of these animals the secretion stains readily with muchæmatein and mucicarmine, applied in the way described above. For the cat and dog, however, I have obtained the most satisfactory stain by employing the eosin-aurantia-indulin mixture of Ehrlich diluted with twenty times its volume of water. By this means the secretion of the glands of Brunner and of the pyloric glands is stained intensely blue, the rest of the tissue red or yellow. The indulin mixture discriminates between the mucin of the glands of Brunner and that of the goblet cells and of the gastric epithelium, and brings out exquisitely the transition in type of the epithelium in passing up the ducts.

In the raccoon, the glands of Brunner extend a distance of about 35 mm. into the duodenum. They form an aggregate of a number of fairly distinct elliptical lobules imbedded in a tela submucosa composed of unusually dense, close-textured, collagenic fibrous tissue. The lobules are confined to the tela submucosa except for the short distance (1.6 mm.) at the beginning of the intestine where a few groups of Brunner's glands (pyloric glands?) occur in the deeper layers of the mucosa among the somewhat dispersed fibers of the lamina muscularis mucosæ.

The lamina muscularis mucosæ is continuous, but perforated here and there by the ducts of the glands of Brunner. For some distance on each side of the sphincter pylori it is composed of longitudinal fibers only, the circular inner layer making its appearance toward the end of the glands of Brunner. From the outer surface of the

lamina muscularis mucosæ smooth muscular fibers radiate into the connective tissue of the tela submucosa, forming a more or less perfect network around the glandular lobules. In the mucous membrane the intestinal epithelium and glands begin just beyond the pyloric sphincter, there being as a rule very little intermingling of intestinal and gastric elements, although for a short distance (8 mm.) a few pyloric glands, serving as ducts for the superficial glands of Brunner, could be discerned.

The lobules are composed of branching tubules of large diameter and lined by large secretion-filled epithelial cells. Each lobule is provided with a duct, often of extraordinary size, which penetrates the lamina muscularis mucosæ, often presenting at this point an ampulla-like dilatation, extends through the mucous membrane a variable distance, and terminates in a shorter or longer gland of Lieberkühn. Rarely these ducts reach the free surface without being intercepted by a gland of Lieberkühn. A minority of the ducts open into the bottoms of the intestinal glands, directly after having passed through the lamina muscularis mucosæ. The majority extend about half the thickness of the mucous membrane, become abruptly reduced in size, and are continued to the surface by a gland of Lieberkühn. A few reach the surface without alteration in the type of epithelial cells. Whenever the ducts open into a gland of Lieberkühn, the change of epithelium is abrupt.

The cells of the glands of Brunner from the single individual available for examination resembled the secretion-filled stage of the opossum's glands. They were from  $16\mu$  to  $22\mu$  in height and filled with secretion. In iron hæmatoxylin preparations the cell exhibited a delicate network with very large meshes, the nucleus being crowded off into one of the basal angles of the cell. A peculiarity of the nuclei in these cells was that, instead of occupying the middle of the base of the cells, they were in one corner and flattened parallel to the lateral walls of the cells. Otherwise the condition was the same as in other secretion-filled mucous cells. *In strong muchæmatein solutions the cells stained intensely and showed a very coarse network of blue-stained trabeculæ.*

No trace of a division of the secretion into two masses was discernible in the cells of the gland tubules, but those of the ducts exhibited a distinct transverse band of cytoplasm, so dividing the secretion.

The resemblance between the cells of the glands of Brunner and those of the pyloric glands in the raccoon is very close, the only character in which a difference could be made out being the subdivision of the secretion into two masses, which was obvious in the pyloric glands, but in the glands of Brunner visible only in the cells of the ducts.

In the mink (*Lutreola vison*), the glands of Brunner are confined to the submucosa and form a compact mass, beginning opposite the pyloric sphincter about 1 mm. beyond the point of greatest thickness of the latter, and extending a distance of about 12 mm. into the duodenum. The glands are composed of more or less rounded lobules closely packed together in the submucosa, which they almost fill from the lamina

muscularis mucosæ to the tunica muscularis. The amount of interlobular connective tissue is relatively small. The zone begins with a number of small scattered elliptical lobules confined to the superficial portion of the tela submucosa, but rapidly increases in width and in number of lobules as the duodenum is descended. The greatest width, 0.7 mm., is reached at a distance of about 5.3 mm. from the beginning of the zone. From this point onward the layer of glands gradually diminishes in thickness until it disappears, so that the whole mass has a fusiform shape in longitudinal section.

The lamina muscularis mucosæ is continuous throughout except at the points where the ducts pass through to enter the tunica mucosa. It consists of a thin internal layer of circular fibers and a thicker external layer of smooth muscle fibers. The structure of the mucous membrane covering the Brunner's glands of the mink is unique, inasmuch as the tunica mucosa of the proximal half of this region, although on the duodenal side of the pyloric sphincter, is covered by gastric epithelium and occupied exclusively by glandulæ pyloricæ identical in structure with those which occur in the stomach itself. These pyloric glands extend into the duodenum a total distance of 8.8 mm. (measured from the summit of the sphincter), slightly beyond the point of greatest thickness of the glands of Brunner. Here they are abruptly succeeded by the intestinal epithelium and glands.

Beyond the point where this change takes place, however, for a distance of 0.3 mm. one may meet pyloric glands mingled with the glands of Lieberkühn, opening into typical foveolæ gastricæ, and may even find patches of gastric epithelium on the surface. There is no lymphatic nodule at the junction of gastric and intestinal mucosæ; both of these mucosæ in this animal are singularly poor in lymphatic tissue.

The glands of Brunner of the proximal half of the zone open directly into branches of the pyloric glands by ducts which pierce the lamina muscularis mucosæ. Beyond the point where the intestinal glands of Lieberkühn make their appearance the glands of Brunner open either into the glands of Lieberkühn or into the pyloric glands with which these are mingled. In the distal parts of the area independent ducts are rare, the glands opening almost exclusively into the bottoms of Lieberkühnian glands. A curious fact in these latter instances is that the epithelium of the proximal branches in the lobule may be lined by cylindrical and goblet cells; in other words, the glands of Lieberkühn, instead of receiving the ducts of the glands of Brunner in the tunica mucosa internal to the lamina muscularis mucosæ, perforate the latter and may subdivide into several branches which retain the typical epithelium before receiving the tubules formed of the epithelium characteristic of the glands of Brunner. We may thus have illustrated in this single animal all the various kinds of ducts between two extremes, one of these being the condition in which independent ducts lined by glandular epithelium and opening into gastric foveolæ are present; the other, that in which the branched glands of Lieberkühn penetrate the tela submucosa and receive the tubules of Brunner's glands.

The tubules of the glands of Brunner in the mink bear a very close resemblance

to those of the cat. They have, as a rule, a rather large lumen surrounded by secreting cells. The average size of the tubules is about  $40\mu$ , but some are as much as  $77\mu$  in width.

The cells vary in width from  $11\mu$  to  $15\mu$ , the two extremes being often found on opposite sides of the same tubule. The cells correspond very closely in structure to those of the opossum; indeed, Fig. 2 would illustrate equally well a typical tubule from the mink. In each cell two well-defined secretion masses may be discerned separated by a transverse band of cytoplasm. *The secretion in these cells stains strongly in muchæmatein applied as indicated above.* The amount of secretion contained in the cells, and so the amount of cytoplasm, vary respectively, in the material examined, with the distance from the sphincter pylori. In the glands near the sphincter, the cells contain a large amount of basal cytoplasm and a spherical nucleus. The proximal mass of secretion is small and stains less intensely in muchæmatein. Going down the zone, the size of the proximal mass progressively increases and encroaches on the basal cytoplasm, the nucleus at the same time becoming correspondingly compressed and crescentic and crowded to the extreme base of the cell.

If we compare the cells of the glands of Brunner of the proximal part of the zone with the cells of the pyloric glands which immediately overlie them, there appears to be an almost perfect similarity of structure. But if we compare Brunner's gland cells from the duodenal end of the zone with pyloric glands several millimeters above the pyloric sphincter, some differences of structure are apparent. In the latter instance the tubules and cells are of about the same size, but in the pyloric glands the cells are to a large extent filled with cytoplasm. The proximal mass of secretion in these upper pyloric-gland cells is scarcely visible, and the distal mass next to the lumen occupies often less than one-fifth of the whole length of the cell. The nuclei are spherical. Again in the pyloric glands as the sphincter is approached the amount of secretion in the gland cells is gradually increased. There is thus in the mink the most perfect transition between the pyloric glands, on the one hand, and the intestinal elements, on the other.

#### IV. THE GLANDS OF BRUNNER OF ERINACEUS

In the European hedgehog the glands of Brunner form a mass of rather large lobules, beginning at the pyloric sphincter and extending a distance of 9.3 mm. into the duodenum. The greatest thickness of the mass is exhibited at the beginning, where the lobules fill the space formed by the sudden falling off of the pyloric sphincter. Here it reaches a thickness of 1.8 mm. From this point it gradually diminishes in thickness to the end of the zone.

The glands are separated from the tunica mucosa by a lamina muscularis mucosæ composed of longitudinal fibers, which form a continuous layer except for a short distance just at the beginning of the duodenum, where the fibers are somewhat inter-

rupted by the ducts of the proximal lobules of the glands of Brunner. At this point the pyloric glands and the glands of Brunner appear in places to be continuous. The dispersed fibers of the lamina muscularis mucosæ radiate out among the lobules of the glands, forming a partial investment for them. At other points a duct which is passing through the muscularis mucosæ may give off a few gland tubules which are thus located among the muscular fibers of the layer.

The ducts of the glands of Brunner in the hedgehog are independent of the glands of Lieberkühn, between which they pass to open on the free surface. A cluster of ducts from the proximal lobules occurs just at the junction of the intestinal and the gastric mucous membrane, some opening between the gastric epithelium on one side and the first villus on the other; the rest, between the bases of the first short villi. In the rest of the zone, the ducts occur at scattered intervals and pass through the mucous membrane without branching or showing a change in type of the epithelium. The glands are composed of richly branching tubules radiating from a central duct in each lobule. The terminal branches are frequently short acinus-like tubules which in fixed preparations, however, seldom show a terminal dilatation.

The tubules are composed of large cells of rectangular or pyramidal shape, surrounding a small lumen. The latter is larger in the tubules which are nearer the main ducts and largest in the ducts themselves. The cells in the specimen from which the descriptions are taken vary in length from  $16\ \mu$  to  $21\ \mu$ , the average being  $18\ \mu$ . The cells in hæmatoxylin sections are clear, transparent, and filled with secretion, between the droplets of which a delicate network may be made out. In the material at the writer's disposal, very few of the cells of the gland tubules exhibit a subdivision of the secretion into two masses. The nuclei are irregular and located at the bases of the cells. In the ducts, on the other hand, the amount of residual protoplasm gradually increases as the surface is approached, and the nucleus expands to an oval shape with the long axis coinciding with that of the cell. In the stronger muchæmatein the contained secretion stains deeply.

The cells of the pyloric glands are very similar to those of the glands of Brunner, although they are somewhat smaller (average length  $15\ \mu$ ) and contain less mucin. Cells of Stöhr are frequent in the pyloric glands, but absent from the glands of Brunner. The secretion in the cells of the pyloric gland, like that of the glands of Brunner, stains with stronger muchæmatein.

#### V. THE GLANDS OF BRUNNER OF THE RODENTIA

Among the rodents it is only in the suborder Myomorpha that the glands of Brunner are confined to the proximal section of the duodenum, between the pylorus and the opening of the ductus communis choledochus. In the rabbit, belonging to the Duplicidentata, the American porcupine, belonging to the Hystricomorpha, and the squirrel, marmot, and gopher (Oppel) belonging to the Sciuiromorpha, they extend far beyond the opening of the bile duct.

In the rabbit, according to Kuczynski (1890), these glands occur in the duodenum as far as the opening of the ductus pancreaticus, a distance of 30 cm.

The glands in the rabbit make their appearance in the tela submucosa at the point where the intestinal epithelium begins. For a distance of about three and one-half centimeters they form two groups, one located in the tunica mucosa, the other in the tela submucosa. These groups are incompletely separated from one another by the lamina muscularis mucosæ, which is interrupted here and there, to permit of communication between the superficial and the deep glands, and the passage of ducts from the latter to the surface. Throughout the rest of the duodenum the glands are for the most part confined to the tela submucosa, although here and there a duct may give off a small group of branches in the tunica mucosa before reaching the submucous group.

The ducts of the superficial lobules of the glands open directly into the glands of Lieberkühn; those belonging to the submucous group preserve their characteristic epithelium for a short distance after passing through the lamina muscularis mucosæ, but are in the majority of cases continued to the free surface by a gland of Lieberkühn. There are, however, a few independent ducts in which the epithelium retains the type characteristic of the glands of Brunner as far as the free surface.

The glands of Brunner of the rabbit are unique in structure, inasmuch as they contain two kinds of cells. This fact was discovered by Schwalbe (1872) who described the more deeply staining elements as independent glands, having the same structure as the pancreas. Schwalbe's observations have been confirmed by Dekhuyzen (1888) and Kuczynski (1890). The latter author showed that the deeply staining elements did not, as Schwalbe thought, form independent glands, but occurred along with the clear cells in the same tubules. Dekhuyzen came to the conclusion that the two kinds of cells represented different functional stages of the same element, and claimed to have seen intermediate types. Castellant (1898) gives an excellent description of the deeply staining cells, in which he observed the small refractive granules of the distal zone and the striation (basal filaments) of the basal zone. He takes exception to the conclusion of Dekhuyzen on the grounds that he could not discover any intermediate stages between the two kinds of cells, and that deeply staining cells are altogether absent in the glands at the beginning of the duodenum. Castellant does not come to any definite conclusion as to the relative specificity of the two kinds of cells, but suggests an interesting comparison between the glands of Brunner of the rabbit and the mixed glands of the trachea.

My own observations confirm the conclusion of Kuczynski that the deeply staining cells occur in the same tubules as the clear cells. The glands of the rabbit are of the compound acinotubular type, the main tubules and some of the terminal acini being composed of clear cells; many of the terminal tubules or acini, of deeply staining cells. The deeply staining cells may occur (*contra* Castellant) in the glands of Brunner at the very beginning of the duodenum.

The transparent tubules are formed by large, clear cuboidal cells surrounding a

lumen  $16\mu$  to  $20\mu$  in width. The clear cells of the glands of Brunner of the rabbit are mucous cells, very similar in type to those of the animals already described. A number of tubules formed by cells of this type is represented in Plate XXI, Fig. 5. The body of the cell presents a coarsely reticular structure, the meshes of which are filled with the reserve secretion of the cell. This secretion may be stained by the use of the stronger muchæmatein, or of mucicarmin, or of Mann's methyl blue-eosin in which it stains blue, or of the eosin-aurantia-indulin mixture recommended by the writer for staining the secretion of the pyloric glands. The appearance of the secretion when so stained differs according to the mode of fixation and subsequent treatment of the tissue. In material fixed in aqueous solutions of corrosive sublimate, the secretion presents the reticular appearance characteristic of mucous cells. The same remark is true of material fixed, without previous removal of the tunica muscularis, in the alcoholic sublimate-bichromate mixture. If, however, the muscular coat of the intestine is removed, and the organ then fixed in the last-mentioned mixture, so as to permit the sublimate and bichromate to come at once into contact with the cells, the secretion is found to be in the form of distinct granules which are, when so fixed, more resistant to the action of water and therefore more permanent in preparations than the corresponding structures in the cells of the opossum. According to the phase of functional activity the secretion is subdivided more or less completely into proximal and distal masses. In Fig. 5, which represents the fully loaded condition, traces of the transverse band of cytoplasm separating the two masses are still visible in most of the cells. The basal cytoplasm is small in amount and contains no basal filaments.

Sections of the fresh tubule examined in blood serum or in salt solution appear perfectly transparent, the granules of secretion corresponding so closely in refractive index to the surrounding medium that they remain invisible.

In material fixed in absolute alcohol and tested by Macallum's microchemical method for the detection of organic compounds of iron, a very faint positive reaction is obtained in the cytoplasm around the nucleus. By Macallum's modification of Lilienfeld and Monti's method for the microchemical detection of phosphorus, the cytoplasm gives a reaction no stronger than that obtained in the cytoplasm of the cylindrical cells of the surface epithelium. The secretion masses remain colorless in this test.

Both in the fresh tissue and in stained sections, the dark tubules present appearances totally different from those just described. In the fresh material examined in blood serum the dark tubules exhibit two well-marked zones. The outer zone is perfectly clear and transparent, no structures whatever being visible in it. The inner zone, on the contrary, is occupied by large numbers of closely packed, minute, highly refractive granules. The appearance of such a fresh tubule is shown in Plate XXI, Fig. 7. The resemblance to a fresh pancreatic tubule or to an active acinus of a serous gland is striking.

In the stained preparations these tubules are distinguished from the mucous tubules by their remarkable staining capacity. The lumen is so small as to be scarcely



visible in many tubules. The cells are of about the same height as the mucous cells, but as a rule narrower and more triangular in outline.

The two zones visible in the living cell are even more obvious in the stained preparations. The basal clear zone of the living cell is distinguished by its great capacity for staining. In hæmatoxylin, carmine, toluidin blue, safranin, and many other nuclear dyes, the basal zone of the cell stains intensely. The substance on which this capacity for staining depends is not evenly distributed in the basal zone, but, as Castellani (1898) has pointed out, gives to the zone an indistinct radially striated appearance (Plate XXI, Fig. 6).

The inner zone of the cell, unless special precautions have been taken to preserve and stain the granules, appears clear with a fine alveolar structure, the spaces corresponding to the granules of the fresh cell, the framework to the cytoplasm separating them.

By Macallum's microchemical test for organic iron, a very intense positive reaction is obtained in the deeply staining substance of the basal or proximal zone. A similarly intense reaction is obtained in this substance, after extraction of the lecithin by alcohol and ether in a Soxhlet apparatus, by the use of Macallum's microchemical reaction for the detection of organic phosphorus. By the latter method a positive result also is obtained in the granules of the inner distal zone.

The microchemical reactions indicate that the substance of the basal zone on which the capacity for basic dyes depends is a nucleo-albumin or nucleoproteid substance, probably the latter, similar to that found in the basal zones of cells from various serous glands, as, for example, the pancreas, the chief cell of the body of the gastric gland, and the cells of the serous glands of the gustatory area of the tongue, the serous cells of the human submaxillary, etc. For this substance, the writer has employed the term "prozymogen," first used by Macallum (1891), to designate a substance which he found in the pancreatic cell by the use of safranin. He afterward (1895) employed the name to designate the iron-holding organic compound of the basal zone of the pancreatic cell, which he identified with the safraninophilous substance of his earlier studies. In 1895 Mouret described the reciprocal relation between the deeply staining filaments of the pancreatic cell previously described by Eberth and Müller (1892), Platner (1889), Macallum (1891), and others, and the zymogen granules, and applied independently of Macallum and without being aware of his work, the name "prezymogen" to the substance of the basal zone. In 1896 Solger described, without attempting an interpretation of them, the deeply staining filaments in the basal zone of the serous cells of the human submaxillary gland, and Erik Müller (1895) observed similar structures in the submaxillary gland of the guinea pig. In the same year I (1896) published a preliminary account of my researches on the gastric glands of vertebrates, in which it was shown by microchemical tests that the so-called basal filaments of serous cells owed their affinity for basic dyes to the fact that they contained a chemical substance similar to chromatin

which was identified as Macallum's prozymogen. In this study it was shown that the prozymogen of the base of the cell increases and diminishes in amount *pari passu* with the diminution and increase respectively of the zymogen granules, and it was therefore regarded as an antecedent substance of the latter. The occurrence of similar substances in the basal zones of the serous cells of the glands of von Ebner and of the oesophageal glands of the frog was also described, and it was suggested that the basal filaments of Solger and Erik Müller were of a similar nature.

Since the publication of this paper, the presence of so-called basal filaments has been demonstrated in a host of serous cells from various sources by Zimmermann (1898), Garnier (1900), Theohari (1899), Cade (1901), and others. The three last mentioned accept in a modified form the interpretation of the writer that the so-called basal filaments contain the antecedent substance of the zymogen granules—a conception which was also adopted by Solger (1899) for his basal filaments of the sub-maxillary gland of man.

It is clear from the foregoing that the dark-staining cells of the rabbit's glands of Brunner agree with serous cells of many other glands in containing large quantities of prozymogen in their outer zones and in containing in their inner zones granules, visible on the fresh cell, of a phosphorus-holding substance, presumably some sort of zymogen. This fact should speedily lead to a chemical examination of the glands of Brunner from this animal to determine the nature of the secretion of these cells. On anatomical and microchemical grounds, there would appear to be a stronger possibility of a positive result in the search for important digestive ferments in the glands of Brunner of this animal than in those of any other mammal.

The conclusion that the dark cells of the glands of Brunner of the rabbit are serous cells is further borne out by the negative evidence afforded by staining in muchæmatein and mucicarmine, in which the secretion granules of these cells remain absolutely colorless.

The facts in connection with the glands of Brunner of the rabbit may be summed up as follows: The glands of Brunner of the rabbit are mixed glands (well compared by Castellant to the mixed glands of the trachea) composed of mucous portions, the cells of which stain strongly in muchæmatein, mucicarmine, etc.; and serous portions, which do not stain in these solutions, but on the contrary possess a basal zone with indistinct radial striation containing a large amount of prozymogen, which may be demonstrated by the microchemical reactions for iron and phosphorus, and an apical zone in which minute granules, presumably of zymogen, are to be seen. These two types of cell are morphologically and chemically distinct from one another, and no intermediate types are to be found.

The pyloric glands of the rabbit are very similar to those of the glands of Brunner, but the former, as Dekhuyzen (1888) pointed out, are, as a rule, smaller and contain more cytoplasm and less secretion than the latter. However, by selecting an animal that has been fasting for some time, pyloric glands may be obtained the cells of

which are indistinguishable by cytological characters from those of the glands of Brunner. In the pyloric glands many cells of Stöhr may be seen. In the glands of Brunner I have failed to find any of these elements.

In the American porcupine the duodenum presents at its beginning a flask-shaped dilatation about 3 cm. in length, into which the common bile duct opens. The glands of Brunner are not, however, confined to this, but extend, as in the rabbit, for a considerable distance into the duodenum. The piece of duodenum available for this examination was 12 cm. in length and throughout contained glands of Brunner. In no part of this area do the glands reach any considerable development. They form a relatively thin layer in the superficial portion of the submucosa, composed of rather small lobules often consisting of a few tubules only. Some tubules may also be seen in the tunica mucosa above the lamina muscularis mucosæ. The glands begin immediately distal to the pyloric sphincter. At this point a well-defined group is present in the mucosa and forms the direct continuation of the pyloric glands of the stomach, although they open either directly, or by means of a gland of Lieberkühn between the villi. Separating these from the small lobules in the submucosa there is a well-defined lamina muscularis mucosæ composed of a thick outer longitudinal stratum and a thin incomplete inner circular layer. In the rest of the region the lamina muscularis mucosæ is thin, composed almost wholly of longitudinal fibers and much interrupted by the passage of ducts. The ducts of the glands of Brunner after passing through the lamina muscularis mucosæ open either directly into the bottoms of the glands of Lieberkühn or ascend as independent ducts for a short distance and open into the sides of the glands of Lieberkühn shortly before reaching the surface. In the latter case the duct is usually joined by a number of tubules which are located in the mucosa. There are also in the mucosa small groups of Brunner's glands which open independently into the sides or bottoms of the glands of Lieberkühn without being connected with those located in the submucosa or with their ducts.

The glandular tubules are formed of cells of a rectangular shape  $8\mu$  to  $17\mu$  in height, the average being about  $13\mu$ . The cells show great uniformity in structure, notwithstanding differences in size. The nucleus has a distinct oxyphile nucleolus, is situated at the base of the cell, and is oval in shape, but is in some cases somewhat flattened or irregular from compression. The body of the cell is transparent in hæmatoxylin preparations. There is but little cytoplasm at the base of the cell, but the secretion is, as in most other mammals, distinctly divided into two masses by a bridge of cytoplasm similar to that illustrated for the opossum in Fig. 2. The proximal mass also, as in the opossum, exhibits a coarser cytoplasmic network than the distal mass. In strong muchæmatein a coarse network, composed of deeply blue-stained trabeculæ, is seen.

The few pyloric glands which are found on the intestinal side of the summit of the pyloric sphincter are exactly similar in all structural details to the glands of Brunner. On the gastric side, however, marked differences are visible which are particularly

apparent in sections stained in muchæmatein. In such preparations the pyloric-gland cells exhibit a very narrow blue-stained margin along the lumen. The proximal mass of secretion in the neighborhood of the nucleus is either wholly absent or only indicated. In the iron hæmatoxylin sections the rest of the cell is found to be filled with cytoplasm free from secretion. The nucleus is full, spherical or oval in outline, rich in chromatin, and separated by a distinct interval from the base of the cell. In size the cell is about the same as the Brunner's gland-cell. The pyloric glands of the porcupine contain numerous cells of Stöhr.

In the guinea pig (*Cavia cobaya*) the glands of Brunner are feebly developed, although they extend a considerable distance into the duodenum, according to Kuczynski (1890) 10 cm. Even at its thickest part, near the sphincter pylori, the layer may be not more than 0.25 mm. in thickness. For a distance of about 7 mm. it forms a fairly continuous layer of thin lobules, but beyond this point the lobules become very small and occur at increasingly greater intervals.\* Each lobule is composed of a cluster of branching tubules connected by a short duct with the bottom of a gland of Lieberkühn.

The tubules are composed of cuboidal to cylindrical or prismatic cells, varying in height from  $9.5\mu$  in the small flattened tubules of the distal lobules to  $14\mu$  to  $18\mu$  in the proximal lobules. The nuclei of these cells are irregularly crescentic in shape and are located in the extreme outer ends of the cells. The body of the cell exhibits the usual transparent reticular appearance when examined in preparations stained in iron hæmatoxylin. There is usually in the middle of the cell a slight condensation of the cytoplasm, a suggestion of the subdivision of the secretion into two masses. In some of the cells, particularly in those of the ducts near the points where they are about to open into the glands of Lieberkühn, and in those forming the tubules of the small distal lobules, a very obvious band of this condensed cytoplasm may stretch across the cell. In the latter case the cytoplasmic trabeculæ which separate the granules of the proximal mass are coarser in texture and form smaller meshes than those of the distal zone. These facts indicate the probability that the mechanism of secretion in the glands of Brunner of the guinea pig is similar to that in the corresponding glands of the opossum and many other mammals.

The cells of the pyloric glands immediately adjacent to the pylorus are exactly similar to those of the glands of Brunner. The glands more remote from the pylorus are formed of wedge-shaped cells  $12.8\mu$  to  $14.3\mu$  in height, surrounding an extremely small lumen. The nuclei of these cells are spherical or oval in shape and located in the base of the cell. The secretion, which stains readily in stronger muchæmatein, occupies a considerable portion of the cell inclosed by the meshes of a cytoplasmic reticulum. In many cells, however, there is a proximal continuous cytoplasmic layer around the nucleus in which may be seen in iron-hæmatoxylin preparations large, coarse, rounded granules, concerning the interpretation of which the writer is in doubt. Perhaps they represent an antecedent substance of the mucin. This is the only

instance in which the writer has seen in the pyloric glands of mammals large granules which are unstainable by mucin stains and which might be confused with zymogen granules. They do not occur in the apical zone of the cell in the midst of the mass of secretion, nor may they be seen in the cells of the glands of Brunner. Similar large granules occur in the mucous cells of the pyloric glands of *Plethodon erythronotus*.

In the ground hog (*Arctomys monax*) the glands of Brunner are direct continuations of the pyloric glands. They begin at the summit of the pyloric sphincter and extend a considerable distance into the intestine—in one specimen throughout the whole piece, 9.5 cm. in length, available for examination. At no point does the glandular layer reach any considerable thickness, the maximum, measured in one specimen, being 0.3 mm. At the beginning of the intestine, the lamina muscularis mucosæ is absent and the glands located in the tunica mucosa and in the tela submucosa respectively form a continuous mass. Farther down the lamina muscularis mucosæ is represented by an interrupted band of longitudinal fibers which subdivides the glands of Brunner into two groups, one located in the submucosa tissue, the other in the mucous membrane. At the lower end of the piece the lobules are small, few and scattered, and are entirely confined to the tela submucosa.

A few of the ducts at the beginning of the zone reach the surface between the villi. Most of them, however, and all of those of the distal portion of the zone, are connected with the surface by means of a gland of Lieberkühn.

The glands are formed of rather large tubules,  $31\ \mu$  to  $61\ \mu$  in width, of which the lumen forms approximately one-third. The tubules branch much less freely than in the other genera already described.

The tubules are formed of large, transparent, secretion-filled cells  $12.7\ \mu$  to  $19.5\ \mu$  in length. The nuclei of these cells are elliptical, in some cells crescentic, in outline, and placed transversely in the proximal ends of the cells. The transparent bodies of the cells exhibit a coarse meshed reticulum composed of cytoplasmic trabeculæ, the meshes of which are filled with the secretion. The latter forms a single continuous mass in each cell, but the similarity in secretory mechanism between this cell and the corresponding cells of the opossum is shown by the differences in character of the cytoplasmic trabeculæ of the proximal and distal portions of the cell. In the proximal portion, the trabeculæ are coarser, with smaller meshes, so that the cell when stained by a strong cytoplasmic stain exhibits two zones. At the junction of these two zones there is a slight concentration of the cytoplasm, probably corresponding to the transverse band which in the intermediate stage of the opossum's cells separates the two masses of secretion.

In the ground hog the cells of the pyloric glands are similar to those of the glands of Brunner. The secretion of both stains readily in the stronger muchæmatein and in mucicarmine. In Mann's methyl blue-eosin the secretion of the pyloric glands, as well as that of the cells of the independent ducts of the glands of Brunner, stains more intensely than that of the glands of Brunner.

As in the marmot, so in the squirrel and gopher (*Spermophilus citillus*) do the glands of Brunner, according to Oppel (1897) extend a considerable distance beyond the point where the common bile duct enters the duodenum.

In the red squirrel (*Sciurus hudsonicus*) I have traced the glands for a distance of 24.6 mm. from the pyloric sphincter. In the specimen from which this measurement was taken the glands, for a distance of 8.3 mm., formed a compact mass, in which separate lobules could not be made out, completely filling the tela submucosa. For a further distance of 6.45 mm. the lobules were distinct, each lobule corresponding to a group of ducts. For the last ten millimeters only scattered small lobules were found, each consisting of a few acini, opening into the bottom of a gland of Lieberkühn.

The glands of Brunner in the squirrel make their appearance at the point opposite the pyloric sphincter where the intestinal epithelium succeeds the gastric epithelium. At this point they are located both in the tunica mucosa and the tela submucosa, the former group being a direct continuation of the pyloric glands. The lamina muscularis mucosæ of the intestine at this point is very imperfect, so that the lobules of the two groups are continuous, the fibers of the muscular lamina being dispersed among the lobules of the glands of Brunner. Beyond the first five millimeters the lobules which are seen in the tunica mucosa are less numerous and are mainly ducts which have subdivided before penetrating into the submucosa.

In one specimen examined by the writer, comprising 10 mm. of the duodenum, the ducts of the glands of Brunner were independent of the glands of Lieberkühn and were lined throughout by cells similar to those of the glandular tubules. In a second specimen, in which the pyloric glands extended a distance of 1 mm. into the duodenum, the proximal group of glands of Brunner opened, together with the pyloric glands, by means of the gastric foveolæ. Beyond the point where the first gland of Lieberkühn made its appearance independent ducts were rare, the ducts opening into the glands of Lieberkühn either as soon as they entered the tunica mucosa, or at various levels between that point and the middle of the layer. The scattered lobules of the lower 10 mm. of the zone opened exclusively into the bottoms of the glands of Lieberkühn.

The cells composing the glands of Brunner in the squirrel are subcylindrical in shape and from  $15.9\mu$  to  $17.2\mu$  in height. The large spherical or elliptical nucleus, placed in the proximal half of the cell, is surrounded by a considerable basal layer of cytoplasm. The inner half of the cell is clear and coarsely reticular. The subdivision of this distal clear segment of the cell into two secondary clear zones by a band of cytoplasm stretching across the cell from side to side is very obvious in many of the cells of the glands of Brunner of the squirrel.

The secretion in the glands of Brunner is very easily stained, even over-ripe solutions of hæmatoxylin giving successful results. In stronger muchæmatein it stains intensely, and in such preparations presents the appearance of a coarse-meshed net-

work. In such muchæmatein preparations the relations of the two masses of secretion may be readily studied. In some of the cells the two masses may be quite distinct; in others there is a deeply staining mass on the free border of the cell, another in the interior, and a faintly staining neck of secretion connecting them. Sometimes the proximal mass is subdivided into two secondary masses, one on each side of the nucleus. In all cases the proximal mass of secretion is closely applied to the surface of the nucleus.

None of the cells in my material was so filled with secretion that the nucleus was flattened by compression.

The cells of the pyloric glands for a short distance (about 6 mm.) above the sphincter are exactly like the glands of Brunner, but exhibit a progressive transition to the type of gland cell which is characteristic of the rest of the pyloric area. In the latter the cells in form and size, as well as in the position and shape of their nuclei, are very similar to the cells of the glands of Brunner. The differences between the cells from the two sources are concerned only with the amount of secretion in the cell. In the pyloric-gland cells the basal cytoplasm extends to very near the free border. The mass of secretion along the free border (distal mass) is much narrower than in the glands of Brunner. The proximal mass in the interior of the cell is represented by a few scattered granules or is absent altogether. The same differences thus occur between the glands of Brunner and the pyloric glands of the stomach as have been already remarked in the mink and the porcupine.

The Myomorpha are represented in the writer's material by the mouse, white rat, dormouse (*Muscardinus avellanarius*), deer mouse (*Peromyscus*), and muskrat (*Fiber zibethicus*). All of these are distinguished from the forms already discussed in this paper by the specialized condition of the stomach. This specialization is carried to the highest degree in the muskrat and deer mouse, in which the gastric glands have disappeared from the whole stomach with the exception of a circular area of fundus glands at the summit of the *curvatura major*, the pyloric glands being represented only by a very narrow zone, in *Fiber* a few millimeters in width, around the pyloric orifice. In *Mus* the specialization is not so great as in *Fiber* and *Peromyscus*, the whole right division of the stomach being occupied by gastric glands. In the dormouse the stomach is also specialized, but as the specialization is of a different kind, the comparison with the other genera as regards its degree cannot be made. In the dormouse this specialization consists in the formation of a bulb-like dilatation containing fundus glands, at the point where the *œsophagus* joins the stomach.

In all these genera the glands of Brunner are of small extent and present obvious differences from the pyloric glands, more particularly in the muskrat, deer mouse, and dormouse.

In the muskrat, the glands begin abruptly on the distal side of the pyloric sphincter as a thick mass completely filling the *tela submucosa* of the intestine and extending into the submucosa underneath the small pyloric-gland area; most of which

is on the distal side of the thickest portion of the sphincter. The lamina muscularis mucosæ is not present as a distinct layer throughout the zone, but is represented by bands of smooth muscle running in various directions among the glands. The glands of Brunner are so closely packed in the submucosa and deeper layers of the mucosa that there is very little division into distinct lobules. The ducts open into the bottoms or sides of the glands of Lieberkühn.

The glands have the usual shape and structure, *i. e.*, are composed of repeatedly branching tubules terminating in elongated pear-shaped acini or short tubules. The whole system of ducts and branches is formed of similar cells.

A transverse section of a tubule of a Brunner's gland from the muskrat is shown, highly magnified, in Plate XXII, Fig. 9. The large lumen is surrounded by somewhat cylindrical cells  $13\mu$  to  $17\mu$  in height, filled with secretion. The secretion is more or less obviously divided into a narrow distal and a larger proximal mass by a band of cytoplasm. The nucleus is flattened or crescentic, and located in the extreme outer end of the cell.

In Plate XXII, Fig. 10 are shown two glands from the pyloric region of the stomach drawn at the same magnification. The differences between this figure and the preceding one are so apparent that they scarcely require comment. The cell of the pyloric gland is much smaller, measuring  $10.5\mu$  to  $11.3\mu$ . The secretion is confined to a narrow band along the free border, and the rest of the cell is occupied by reticular cytoplasm containing an oval nucleus rich in chromatin. Several cells of Stöhr may be seen.

The secretion contained in the cells of the glands of Brunner and of the pyloric glands stains readily in strong muchæmatein; that of the glands of Brunner staining the deeper color. That this difference in the intensity of the staining does not mean a greater concentration of the mucin in the two cells is indicated by the result obtained in sections stained with Mann's methyl blue-eosin. In this the secretion contained in the cells of the pyloric glands stains deep blue, that of the glands of Brunner pale blue.

In the deer mouse the extent of the glands of Brunner, measured in one specimen, was 2.6 mm. The cells of the glands differed from those of the muskrat in the specimen examined in that the proximal mass of secretion was less compact than the distal mass, and the segment of the cell in which it was located contained relatively more cytoplasm. There was in addition a narrow basal layer of cytoplasm containing the slightly flattened oval nucleus. The cells of the pyloric glands differed from those of Brunner's glands in this animal in much the same way as in the muskrat. The pyloric-gland cells were very small, had a relatively large oval nucleus, and contained but a small amount of stored-up secretion. The secretion of both kinds of cells stained readily in stronger muchæmatein.

In the dormouse examined (*Muscardinus avellanarius*) the glands extended a distance of 3.5 mm. into the intestine. Throughout this region the lamina muscularis mucosæ was represented only by scattered fibers. The glands formed a thin continu-



ous layer in the submucosa, except at the end of the zone, where the glands terminated as scattered small lobules. The ducts emptied into the glands of Lieberkühn.

The differences between the cells of the glands of Brunner and those of the pyloric glands were greater in this animal than in any other mammal examined. The former cells were large, transparent, 15–17 $\mu$  in height, completely filled with secretion without any indication of subdivision into two masses. The nucleus was crescentic and placed in the base of the cell. The cells of the pyloric glands were small, 9 $\mu$  in height, contained very little secretion, and possessed a relatively large oval nucleus. The secretion contained in both kinds of cells stained readily in muchæmatein and mucicarmine.

In the white rat the glands of Brunner begin abruptly as a mass of considerable thickness occupying the space formed by the sudden falling off in thickness of the muscular coat on the dorsal side of the sphincter pylori. According to Kuczynski (1890), they extend into the duodenum a distance of 4.2 to 9 mm. In one specimen measured by the writer the extent was 5.5 mm. The greatest thickness of the mass is exhibited at the very beginning of the zone, where they reach a thickness of 1 mm. From this point onward the zone rapidly diminishes in thickness and, beyond a point 3 mm. to 3.5 mm. from the sphincter, is only represented by scattered small lobules. Throughout the zone the lamina muscularis mucosæ is defective. The glands discharge into the glands of Lieberkühn.

The cells of the glands of Brunner of the rat have recently been the subject of a careful study by Castellant. This author finds that the glands of Brunner of the rat present no anatomical relation to the pyloric glands and that the study of their fine structure places them still farther apart. He describes the cells as follows: "*Leurs cellules sécrétantes, de forme pyramidale présentent un contenu divisé en deux zones; l'une basale, granuleuse, où se trouve le noyau; l'autre, apicale qui reste claire, quelque soit le liquide fixateur employé, alcool, liquide de Flemming, acide osmique.*" He found that staining with hæmatoxylin after Flemming's fluid did not color the clear zone of the glands of Brunner. Mayer's mucicarmine also, in Castellant's hands, gave negative results, but thionin staining after treatment with acetic acid gave a faint reddish color which he thought might be interpreted as revealing the presence of a little mucin.

Castellant also studied the mechanism of secretion in the cells of the glands of Brunner of the rat in preparations fixed in Flemming's fluid. He found that for the first two hours of digestion these cells increase in size, the apical clear zone increases in size, and the basal granular zone is diminished in amount. From the third hour of digestion onward he found a progressive reduction in the amount of secretion in the cell to the end of the seventh hour, when the cell is almost wholly granular and contains little secretion.

Castellant does not specify the exact nature of the differences which he found between the pyloric gland cells and those of Brunner's glands, but it may be inferred that the clear subdivision of the cell into two zones in the glands of Brunner is one of them.

The use of stronger muchæmatein which stains intensely the secretion of the cells both of the glands of Brunner and of the pyloric glands of the rat has enabled me to extend somewhat the description given by Castellant. As the latter points out, the distal (apical) zone of the cell in the rat is remarkable for the extreme tenuity of the fibers of the cytoplasmic network which it contains. It does not, however, contain the whole of the secretion of the cell. In sections stained in muchæmatein it may be seen that even cells in the resting condition, in which a large proximal mass of cytoplasm is visible, may contain small granules of stainable secretion, in the portion of this cytoplasm between the nucleus and the deeply stained secretion-filled distal zone of the cell. In some of the cells these granules have so increased in number that the places occupied by them are recognizable in hæmatoxylin-eosin preparations as clear spaces in this portion of the cell. This appearance marks the transition phase from the resting condition of Castellant's observations to the more loaded condition of the third hour of digestion, and corresponds exactly to what I (1898) have found to be the case under similar experimental conditions in the pyloric glands of the cat. The explanation is that the cell when it passes from the resting to the active phase begins to transform rapidly into mucigen the reserve material contained in its basal cytoplasm, the product of this transformation making its appearance in the space between the old secretion and the nucleus. During the first hours of digestion this transformation of antecedent substance into mucigen goes on more rapidly than either the secretion of the mucin from the cell or the repair of the basal cytoplasm from which it is formed. The result is the increase in reserve secretion in the cell. A cell in this condition of maximum loading presents three distinct zones; a narrow basal zone of protoplasm containing the now slightly flattened nucleus; then the proximal mass of secretion, subdivided by coarse trabeculæ of cytoplasm; then the third zone, with which it is continuous and into which it passes by gradual transition. The third zone contains the distal mass of secretion along the free border of the cell and is remarkable for the extreme delicacy of the cytoplasmic threads which penetrate it.

The cells of the glands of Brunner of the rat may therefore present one of three conditions according to the phase of activity. Either there is one narrow mass of secretion along the free border; or there are two distinct masses of secretion, one along the free border and one in the interior of the cell; or, finally, there is a single continuous mass of secretion which shows evidences in the structure of the included cytoplasmic trabeculæ of its having been produced by the fusion of two masses originally distinct.

The cells of the pyloric glands of the rat differ from those of the glands of Brunner in the same way as do the corresponding structures in the muskrat, dormouse, and deer mouse. The cells of the glands of Brunner are from  $13.3\mu$  to  $17.2\mu$ , and those of the pyloric glands from  $8.9\mu$  to  $10.8\mu$  in height, although an occasional cell may reach a height of  $13\mu$ . The averages are  $15.7\mu$  for the glands of Brunner, and  $9\mu$  for the pyloric glands. The differences of size are less obvious when both glands are in the fully loaded condition.

The secretion of the glands of the pyloric region forms a narrow band along the lumen which is much more compact in structure than the secretion in the glands of Brunner, and like the contents of the theca in the gastric epithelium cell, retains some color in sections stained in iron hæmatoxylin, in which the secretion of the Brunner's glands are colorless. It also stains more intensely blue in Mann's methyl blue-eosin. The basal cytoplasm is more granular in appearance, and the oval nucleus is larger and richer in chromatin, than in the cells of the glands of Brunner.

The glands of Brunner and the pyloric glands of the mouse correspond so closely in structure and staining reactions with those of the rat, that they do not call for special description.

#### VI. THE GLANDS OF BRUNNER OF THE ARTIODACTYLA

The topography of the glands of Brunner of the Ungulates has been recently studied by Hock (1899). In the horse he found the glands to extend the enormous distance of seven meters into the intestine. In the pig and the sheep—two of the four genera of the Artiodactyla examined by him—they were also of considerable extent: 40 cm. in the six-weeks-old pig, and between 30 cm. and 40 cm. in the sheep. In a young goat two or three weeks old he found the glands to extend only 4 cm. into the duodenum. For the ox he gives no measurements.

In my studies the Artiodactyla are represented by the sheep and pig, which are especially interesting because they represent the two extremes of specialization of the stomach of the recent Artiodactyla, and because they present differences in the relation of the pyloric glands to the glands of Brunner which may be compared to those shown by the Rodentia with simple stomachs and complex stomachs respectively. In the pig, in which the stomach presents the simplest form found in the group, the two kinds of glands resemble one another very closely; in the sheep, with a highly specialized stomach, the two kinds of glands are very different. They are further of interest because of Kuczynski's failure to stain the secretion by any of the methods he employed.

As far as the arrangement and topography of the glands are concerned, Hock's excellent description leaves little to be desired. In the pig, according to him, the glands of Brunner are the direct continuation of the enormously developed pyloric glands, which at the summit of the pyloric sphincter begin to divide more freely and extend more deeply, forming new lobules which completely fill the tunica mucosa and open by short tortuous ducts into the foveolæ gastricæ. Presently they exceed the limits of the mucosa and extend over into and fill the submucosa. For about  $1\frac{1}{2}$  cm., according to Hock, the lamina muscularis mucosæ is not present as a distinct layer, but the fibers are dispersed among the lobules of the glands, to the muscular investment of which the pyloric sphincter also contributes fibers. For a distance of 20 cm. from the pylorus the glands are described as forming a compact mass, completely filling the submucosa. He finds that the ducts at the beginning of the zone discharge into the gastric foveolæ; beyond the point of the first appearance of the glandulæ

intestinales of Lieberkühn, they empty almost exclusively into the latter. A few independent ducts, however, are present.

The large glandular tubules are composed of large cylindrical cells,  $18\mu$ – $21\mu$  in height, surrounding a very narrow lumen. The nucleus is flattened or crescentic and placed in the base of the cell. The body of the cell exhibits a faintly staining network containing the secretion. Kuczynski (1890) was unable to obtain any specific staining of this secretion by the methods he employed. He was also unsuccessful in attempting to stain the secretion of the similar cells of the pyloric glands, although he remarks that the pyloric glands near the fundus zone stain with Victoria blue. He concluded that if they contain any mucin it was not their exclusive constituent. By the technique recommended at the beginning of this article, I have succeeded in staining the secretion of both the pyloric glands and the glands of Brunner intensely in muchæmatein and in mucicarmine. When so stained the meshes of the cytoplasmic network of the body of the cell are found to be filled with a compact mass of small granules. Staining in indulin-eosin-aurantia mixture also gave successful results. There is, therefore, no adequate reason for supposing that the cells of the glands of Brunner of the pig are essentially different in function from those of other mammals.

In the sheep the differences between the glands of Brunner and the pyloric glands are very striking, and I have found that even with the solutions which I have employed with success on other mammals the secretion of the Brunner's glands stains with difficulty.

The pyloric glands of the sheep are simple tubes composed of somewhat narrow triangular cells  $12.5\mu$  to  $17\mu$  in height with nuclei round, oval, or crescentic according to the shape and secretory condition of the cell. Around the nucleus is a very small amount of finely reticular cytoplasm. The body of the cell is transparent, and finely reticular. The secretion forms a continuous mass, and stains readily and deeply in muchæmatein and mucicarmine.

The glands of Brunner are exceedingly large tubules, with wide lumina surrounded by cylindrical cells  $16\mu$  to  $22\mu$  in height. The nucleus in these cells is spherical or oval, slightly cupped on the side directed toward the lumen, and located in the proximal end of the cell. The whole of the cell between the nucleus and the lumen is filled with secretion which is distinctly divided into a proximal and distal mass in many of the cells. The secretion stains in muchæmatein, but much more slowly, and less intensely, than that of the pyloric glands. The differences in shape and character of the tubules and cells of the glands of Brunner are particularly obvious opposite the sphincter pylori where glands of Brunner occur in the mucous membrane side by side with the pyloric glands. Very striking at this point is the contrast, in muchæmatein preparations, between the deeply stained narrow tubules of the pyloric glands and the faintly stained wide tubules of the glands of Brunner. The difference in staining capacity does not, as will be pointed out later, imply a difference in the amount of mucin secreted by the cells.

## VII. THE GLANDS OF BRUNNER OF MAN

The material for this study consisted of the duodenum and a portion of the jejunum of an executed criminal, a young man about thirty years of age. The material was obtained about forty-five minutes after death and, although the epithelium of the free surface and portions of the villi were lost in places, proved to be in other respects excellently preserved. A strip from end to end of the duodenum was fixed in alcoholic sublimate bichromate mixture and the rest in 70 per cent. alcohol. For comparison a second duodenum obtained for the writer from the body of a woman seventy years of age, and fixed in alcoholic sublimate bichromate, was studied. In the latter marked cell-atrophy was exhibited by the glands of Brunner, but some interesting facts were obtained as to the mode of accumulation of the secretion in the cell.

The observations of the writer confirm in the main those of Renaut (1879), Schaffer (1891), and Castellant (1898) as to the distribution of the glands of Brunner in the intestine of man.

The glands make their appearance in the mucosa and submucosa, opposite the summit of the pyloric sphincter, at the point where the first intestinal gland of Lieberkühn appears. There is for a short distance, about 2.5 mm. in my material, a slight mingling of intestinal and gastric glands as observed by Böhm and von Davidoff (1895), and confirmed by Castellant (1898). In this region, however, only a few pyloric glands are visible, and in some of these goblet cells occur among the gastric epithelial cells.

The glands of Brunner located in the mucous membrane at this point form groups of radiating, slightly wavy, branched tubules, clustered around the base of a gland of Lieberkühn, or of a foveola gastrica into which they open. They are not in this region clearly marked off into lobules, and are a direct continuation of the pyloric glands which, as many writers have pointed out, exhibit a tendency to richer branching near the beginning of the duodenum.

In the pars superior of the duodenum from the first individual the surface of the mucosa presented a somewhat mammillated appearance, owing to the occurrence in it of many large solitary follicles (*noduli lymphatici solitarii*). In sections these solitary follicles form interruptions at regular intervals in the continuity of the glandular elements of the mucous membrane, as may be seen in Plate XXIV, Figs. 14 and 15. This fact gives in sections an appearance of regular grouping of the glands located in the tela submucosa as well as of those in the mucous membrane, because, obviously, the ducts of the former must open to the surface between the solitary follicles and the lobules of the glands must be arranged in a radial fashion around the ducts. This appearance is well illustrated in Fig. 15, where the lobules of the group in the submucosa spread out in a fan-like fashion from the point in the mucosa where their terminal ducts are located.

In the pars superior duodeni the glands of Brunner, as described by Renaut (1879) and confirmed by Schaffer (1891) and Castellant (1898), form two groups,

one located in the mucous membrane, the other in the submucosa. The former, except for the interruptions caused by the lymphatic nodules, form an almost continuous layer occupying exclusively the outer half of the mucous membrane, the inner half being occupied by the glands of Lieberkühn and the villi. The lobulation is not very striking, although, as shown in Fig. 14, the groups of glands which empty into a single duct may be easily recognized. These groups are somewhat elliptical or triangular in section, the long axes being perpendicular to the surface of the mucous membrane. From the inner end of each group one, two, or more ducts emerge, which either join to form a single duct, or open separately into the same gland of Lieberkühn or into side branches of the latter.

Each group is composed of a cluster of tubules, which are the primary branches of the duct, and of the numerous ramifications of the latter. The most prevalent mode of branching is as follows: the duct divides near its origin into a tassel-like group of wavy tubules which pass outward in the direction of the lamina muscularis mucosæ. Each of these tubules gives off on all sides radial descending branches, which in their turn, after a very short course, subdivide and terminate either as short tubules of the same diameter as the parent tubules, or as slightly expanded, elongated, pear-shaped acini. Some of the groups extend through the interrupted muscularis mucosæ to form lobules or groups of lobules in the inner layer of the tela submucosa.

The group of glands in the submucosa is composed of elliptical and fusiform lobules of small size, placed with their long axes nearly parallel to the free surface of the duodenum. These lobules are confined to the inner layer of the tela submucosa, there being usually an outer layer nearest the tunica muscularis free from glands and containing aggregations of adipose tissue. The lobules are not all independent of one another, several being often strung out along the same tortuous duct. Again, many of the larger lobules are subdivided into marginal lappets which represent the groups of branches clustered around each of the radial branches of the principal duct of the lobule.

The lobules of the submucous group of glands are derived from three sources. Some consist of the continuation into the submucosa of groups of tubules, the main bulk of which is located in the mucous membrane. In a second series a tubule, after giving off a number of branches in the mucous membrane, passes through an aperture in the lamina muscularis mucosæ, and gives rise to one or several lobules in the submucosa. In the third series a duct of variable size passes directly from the bottom or side of a gland of Lieberkühn to the submucosa, where it terminates in a lobule or a series of lobules. In the pars superior duodeni of the cases examined the second, in the pars horizontalis and pars ascendens of the organ the third, was the prevailing type.

The point of junction with the gland of Lieberkühn is a variable one, but I have not observed, in either of the two duodena examined, a single instance of the duct reaching the free surface independently of the intestinal glands, although that such cases do occur, is shown by the observations of Schaffer and Castellant.

As regards the distribution of the glands of Brunner in man, the observations of

the writer confirm the description given by Castellant (1898), except with respect to their downward extent. In this case the glands extend only within about 3.5 cm. of the duodenojejunal flexure; in Castellant's case isolated lobules were found in the upper part of the jejunum.

From the beginning of the pars descendens duodeni downward the glands are progressively reduced in bulk and tend, as Castellant observed, to become located in the plicæ circulares (Kerkringi), although not wholly confined to the latter. In the lower part of the duodenum they are reduced to scattered small lobules. Throughout, however, there are tubules in the tunica mucosa as well as in the submucosa, although the latter predominate. Castellant remarks concerning these tubules of the mucous membrane: "Elles cessent même presque complètement au niveau de l'union de la première portion du duodénum avec la seconde; on n'en retrouve plus qu'accidentellement au delà"—indicating that he observed them in the lower portion of the duodenum, but in smaller numbers than in the material described above.

In each of the lobules of the submucous group may be distinguished a central tubule which is the duct of the lobule. From this duct, which may be extremely tortuous in its course, come off numerous side branches of various lengths and complexity of secondary branching. After passing through one lobule, a duct may enter a second and a third, branching in each in a similar way; or some of the side branches may pass out and form the central duct of accessory lobules. Often the central duct of a lobule may be locally enlarged, as may be seen in some parts of Fig. 15.

The ducts pass through openings in the muscularis mucosæ sometimes singly, more often in groups, and empty into the bottoms, sides, or branches of the glands of Lieberkühn. In the submucous as in the mucous group, the terminal branches of the secreting tubules are short tubules of the same size as the main branches, or elongated, pear-shaped acini. In both cases they are formed of cells similar to those forming the ducts and their various branches.

All the tubules of the glands of Brunner of man are provided with a delicate basement membrane composed of reticulum. The ducts and tubules are formed of somewhat rectangular epithelial cells,  $15\mu$  to  $21\mu$  in height, uniform in type throughout the gland, but with some differences of structural details in different parts. In the terminal tubules in the material from the young subject, the cells are of the type represented in Plate XXII, Figs. 11 and 12. The nucleus is crescentic or flattened in form, and is located in the outer end of the cell. The body of the cell contains a network composed of extremely fine cytoplasmic fibrils forming large meshes in which the secretion of the cell is contained. In the center of the cell the cytoplasm forms a network of smaller meshes and coarser trabeculae corresponding to the band of cytoplasm separating the two masses of secretion in the cells of the opossum, cat, mink, etc.

As may be seen from Figs. 14 and 15, which are half-tone reproductions of photomicrographs of specimens stained in strong muchæmatein, the glands of Brunner stain even more intensely in this solution than the goblet cells of the

intestinal glands. In strong mucicarmine a similar result is obtained. The appearance of the stored-up secretion when stained in muchæmatein depends on the mode of fixation and subsequent treatment. In the material fixed in alcoholic bichromate sublimate, imbedded in celloidin, sectioned and stained, without passing through water, the secretion is in the form of minute granules of smaller size and less closely packed than those in cells from the glands of Brunner of the opossum. In sections cut in paraffin, fastened to the slide by the water method, and stained with muchæmatein, the secretion presents itself in the cell in the form of a coarse-meshed network. A result similar to the latter is obtained with material fixed in 70 per cent. alcohol, except that the meshes of the mucin network are much larger and are formed of thicker trabeculæ.

In the ducts the cells are similar to those in the tubules and acini; indeed, many of the ducts of the small lobules are indistinguishable from the other tubules forming the lobules except by the method of tracing them out in serial sections. In the larger ducts, however, and particularly in locally dilated portions of them, the cells, while similar to those of the acini, tend to be more protoplasmic in nature. In such cells the division of the secretion into two masses and the transverse band of cytoplasm separating them are particularly obvious, and the cell presents a structure exactly comparable to that shown in Fig. 2, which is taken from the cells of the opossum. Zimmermann (1898) described and figured this condition in the cells of the glands of Brunner of man, but did not recognize the fact that the outer clear zone near the nucleus represented a second accumulation of secretion in that part of the cell.

In passing from a duct to a side branch of it, there is a gradual transition from this more cytoplasmic type of cell with spherical nucleus and two distinct masses of secretion to the secretion-filled cell with a single continuous mass and crescentic nucleus.

A similar transition occurs in the tubules of the glands located in the mucosa. A small gland from this source, together with a portion of the gland of Lieberkühn into which it opens, is shown in Fig. 12. In the gland of Lieberkühn in this figure the three typical elements—cylindrical cells, goblet cells, and one Paneth cell—are seen. The change to the epithelium of Brunner's gland, as indicated by Schaffer (1898), is abrupt, *i. e.*, there are no intermediate stages between it and the intestinal epithelial elements. In the Brunner's gland, however, a gradual transition is to be observed from the cell with a narrow band of secretion along the lumen, a large mass of cytoplasm, and a spherical nucleus, to the secretion-filled cell with a crescentic, basally situated nucleus. In some of the latter the remains of the band of cytoplasm separating the two primary masses of secretion may be clearly seen.

A point of considerable interest is the occurrence in the human glands of Brunner of a very small number of parietal cells exactly similar to those seen in the gastric glands. These cells occur in very small numbers, but their structure is so characteristic that there can be no doubt of the correctness of their identification. They



contain distinct intracellular ducts, and exhibit the three characteristic zones of structures described by Zimmermann (1898) for the parietal cells of the stomach. As in the latter the nuclei may be multiple.

The cells of the pyloric glands in man resemble very closely in size and structure those of the glands of Brunner. In general, in the former the subdivision of the secretion into two masses is more obvious and the proximal mass contains coarser cytoplasmic trabeculae.

The cells of the glands of Brunner of the old subject were, as has already been remarked, considerably atrophied. They measured  $12\mu$  to  $14\mu$  in height. The lumen showed a corresponding enlargement. In most of the tubules the secretion stainable in strong muchæmatein was confined to a narrow zone less than  $2\mu$  in width along the lumen. The rest of the cell was occupied by a continuous mass of cytoplasm containing the oval nucleus. In some of the cells, however, a second small proximal mass of secretion occurred in the midst of this cytoplasm between the nucleus and the narrow distal mass, and in a few tubules the two had become confluent, forming a single large mass of secretion, filling all of the space between the now flattened nucleus and the lumen.

#### VIII. DISCUSSION OF RESULTS

The results of this investigation show that there is a remarkable uniformity in the nature and structure of the glands of Brunner of many mammals. In eighteen out of the nineteen genera examined the glands are of the pure mucous type. This conclusion is based on the structure of the cells of the glands, and on their staining and microchemical properties. The evidence, which is partly negative, partly positive, may be briefly summed up as follows:

The cells of the glands, when examined fresh in serum or normal salt solution, do not show easily visible secretion-granules. The granules (droplets?) of secretion like those of known mucous glands correspond so closely in refractive power with the mounting media that they are almost invisible.

The cells when fixed and stained do not contain basal filaments (prozymogen), and the microchemical test for organic iron indicates the presence of only a relatively small amount of cytoplasmic nucleoproteid. Serous cells from other sources, on the other hand, show the presence of a large amount of cytoplasmic nucleoproteid, either in the form of the so-called basal filaments (composed largely of the nucleoproteid prozymogen) or in the form of prozymogen diffused in the basal cytoplasm.

The granules of zymogen in the pancreatic and gastric ferment-secreting cells stain strongly in iron hæmatoxylin. The secretion of the cells of the glands of Brunner remain colorless in this stain.

A more positive and selective stain for the granules of zymogen of the stomach and pancreas, Reinke's neutral gentian, as modified by the writer (1900), also gives negative results with the cells of the glands of Brunner.

The secretion granules of the cells of the glands of Brunner give no reaction when tested by Macallum's method for the microchemical detection of organic phosphorus. The progress of this reaction is easily controlled, in the case of the glands of Brunner, by observing the effect on the granules in adherent sections of the pancreas, which give a positive reaction for organic phosphorus after treatment for two hours with nitric acid ammonium molybdate. The results with this test in the glands of Brunner, with the exception of the dark tubules of the glands in the rabbit, are absolutely negative.

Not only do the cells of Brunner not contain the chemical substances which it is possible to recognize in the serous cell either by examining the fresh cell or by employing staining and microchemical methods, but, on the contrary, there is positive evidence that they contain something else, which we have good reason to believe is mucin.

The secretion contained in the cells of the glands of Brunner stains with uniform facility, if certain precautions are taken, in Mayer's alcoholic muchæmatein and mucicarmine. With muchæmatein the precautions necessary to success are as follows:

If the dilute solution recommended by Mayer is employed, the sections cut in paraffin should be transferred, *without attaching them to slides*, to benzole, thence to absolute alcohol, thence directly to the staining solution. After five minutes in the latter, they are washed with 70 per cent. alcohol, dehydrated, cleared, and mounted.

A stronger solution (having the following formula: hæmatein, 1 g., aluminium chloride, 0.5 g., 70 per cent. alcohol, 100 c.c.) gives better results, and can be used on sections fastened to the slides or on celloidin sections with certainty of speedy and satisfactory results. The degree of acidity of this solution is of some importance. The writer is in the habit of reducing the acidity by diluting his alcohol with tap water containing calcium bicarbonate. After the solution so prepared has stood for a week, it is tested on a section. If the resulting stain is slightly diffuse, a 10 per cent. solution of nitric acid is added, a drop at a time, the staining properties being tested on a section after the addition of each drop of acid. This is kept up until the correct reaction is obtained. The solution so prepared is employed as follows:

The sections cut in paraffin and fastened to the slide by the albumen or by the water method are treated with benzole followed by absolute alcohol. The slide having been placed on the stage of the microscope, a drop of the staining solution is applied to the section, and the latter is watched under the microscope until a proper depth of color is obtained in the secretion within the cells. It is then rapidly washed in 70 per cent. alcohol, dehydrated, cleared in benzole, and mounted in benzol balsam.

If the staining is prolonged and the solution is not renewed from time to time, the sections after attaining a maximum depth of color will slowly fade out again, probably owing to reduction in the acidity of the solution by the absorption of ammonia from the atmosphere. Sections should not be washed in water after staining, as this procedure completely removes the stain from the mucous cells.

This stronger muchæmatein solution *stains deep blue* the secretion contained in

cells from the following sources: mucous cells from the submaxillary, sublingual, lingual, palatine, tracheal, and oesophageal glands; the gastric epithelial cells; the cells of the cardiac glands of the stomach; the cells of the pyloric glands; the neck chief cells of the fundus glands of the stomach; goblet cells; and the cells of the glands of Brunner (except the dark tubules of the rabbit's glands). *It does not stain* the secretion in cells from the following sources; demilune cells of the salivary glands; the cells of the parotid gland; the serous cells of the submaxillary or sublingual glands; the serous portions of the palatine glands and tracheal glands; nor the ferment-forming cells of the pancreas and of the fundus glands of the stomach.

An idea of the intensity of the resulting color got by this method may be obtained from Figs. 14 and 15, which are half-tone reproductions of photomicrographs of sections of the human glands of Brunner stained in stronger muchæmatein. An equally strong stain was obtained in the glands of Brunner of all the animals examined, with the single exception of the sheep, in which the secretion stained positively, but more slowly and with less intensity.

For mucicarmine the conditions of success are that the solution be employed undiluted in the form of Mayer's stock solution, and that it be freshly prepared. In the writer's hands the solution, after twenty-four to forty-eight hours, refuses to stain and cannot be filtered. The solution is to be applied in exactly the same way as muchæmatein and gives similar results, both on the glands of Brunner and on the other glands mentioned. In view of the results in the several glands of known character which are enumerated above, we are justified in concluding that the solutions employed as recommended do stain mucous cells and do not stain serous (zymogenic) cells.

In view of Mayer's observation that the clear cells of the submaxillary gland of the hedgehog, which do not secrete mucin, stain with muchæmatein, some conservatism must, however, be exercised in interpreting the results. It is obvious that no absolute proof of the mucous character of the glands of Brunner can be brought forward until a positive microchemical test for the various mucins is devised, or until some one undertakes and completes the laborious task of isolating the lobules of Brunner's glands carefully by dissection and studying them by the ordinary macrochemical methods. The mucous nature of the glands is, however, supported by the recent work of Ponomareff (1902) in Pawlow's laboratory, who isolated a portion of the duodenum by Thiry's method as modified by Pawlow. The juice obtained was colorless, thick, and very viscid.

Further evidence is, however, afforded by the tests applied to determine the solubility of the secretion in various solutions. This is accomplished by using muchæmatein as an indicator. The sections are fastened to the slide by the water method, and are placed in the solution to be tested. From time to time a section is taken out, washed thoroughly, and stained by muchæmatein. By this means it has been determined that the contents of the cells of the glands of Brunner of the opossum and of man are soluble in weak alkaline solution, insoluble in 5 per cent. solution of hydro-

chloric acid and in artificial gastric juice containing 0.2 per cent. of hydrochloric acid.

The structure of the cells also supports the conclusion that they are mucous cells.

The cells of the glands of Brunner differ in structure according to the physiological phase in which they happen to be when examined. Three well-defined stages may be discerned: In the condition of maximum loading the cells are large and transparent. They contain a flattened or crescentic nucleus, located in the base of the cell, surrounded by a small quantity of finely reticular cytoplasm. The body of the cell is clear and shows a coarse network of cytoplasmic trabeculæ in the meshes of which the secretion is lodged.

In the intermediate condition, the nucleus is more oval in outline, the basal cytoplasm is greater in amount and the body of the cell exhibits two distinct secretory zones. In the proximal zone the granules of secretion are separated from one another by cytoplasmic trabeculæ coarser than those of the distal zone. (See Fig. 2.)

In the discharged condition the nucleus is spherical or oval and nearer the center of the cell. The basal cytoplasm is increased in amount. The secretion may be confined to a mass on the free border of the cell, or there may be two masses, a dense one on the free border (referred to in the specific descriptions as the distal mass) and a less dense one (the proximal mass) composed of smaller granules, in the interior of the cell.

The two latter conditions may be reached either as stages in the discharge of the cell, or in inverse order, as stages in its recovery during a period of rest after discharge of its secretion.

In the writer's opinion, the obvious subdivision of the secretion into two masses is due to the fact that the new secretion is formed in the neighborhood of the nucleus in the interior of the cell. This may be due, as suggested above, to the action of enzymes produced by the nucleus, or it may be due to the effect of the presence (of which the writer has not yet been able to obtain evidence) in these cells of structures similar to the so-called trophospongium observed by Holmgren (1902) in various epithelial cells.

A similar secretory mechanism has been shown to exist in the various mucous salivary glands by the studies of Maximow (1901) and Kolossow (1903). Both of these writers have noticed the obvious division of the secretory portion of mucous cells into two zones, and Maximow has also observed the new formation of secretion granules in the neighborhood of the nucleus. I have (1898, 1902) demonstrated similar conditions in the cells of the palatine glands, pyloric glands, cardiac glands, and in the mucous neck chief cells of the fundus glands of mammals. Krause (1895) has also described the formation of new secretion near the nucleus in the mucous cells of the retrolingual glands of *Erinaceus*.

The conclusion that the glands of Brunner are mucous glands is concurred in by a number of writers mentioned in the introductory paragraphs. Castellant (1898), Kuczynski (1890), and Schaffer (1891) came to a similar conclusion, with some reservations. The two former, using various synthetic stains and ordinary solutions of hæmatoxylin, have observed that the secretion of the glands of Brunner of different

mammals, and indeed of different tubules of the same animal, stain with different degrees of facility. They conclude that the depth of staining indicates the amount of mucin present in the cell, and that some cells contained a great deal of mucin, others a little, and still others none at all. The uniformly intense stain obtained by the writer by means of his muchæmatein technique shows that this conclusion is not justified. The cells are to all appearance in all mammals equally engaged in mucin secretion.

Does the different capacity for staining in these synthetic dyes indicate, as Schaffer (1891) thought, a difference in the nature of the mucins formed? It is, of course, possible that this is the case. We already know many different mucins, and we have reason to suspect, as Huppert (1896) has suggested, that there is a great number of different glycoproteids. Although it is possible that different mucins are secreted by the glands of Brunner in different mammals, it does not appear to me to be necessary to assume that this is the case in order to explain the different staining properties.

It is known that the mucous cells do not store their secretion as mucin, but as a substance (mucigen) which may readily be transformed into mucin. It is not possible definitely to identify the granules visible in the cell as mucigen, but they are probably composed of one of the antecedents of mucin, and not of mucin itself. It is, moreover, probable that the transformation of the substances received by the cell into mucin is not accomplished by one or even two steps, but that there are many stages in this chemical process. Furthermore, it is a well-known fact that similar cells from different sources, engaged in the formation of the same product, may store it in the form of different antecedent substances. For example, the chief cells of the glands of the gastric fundus of the rabbit in the resting condition are filled with zymogen granules and contain little prozymogen. The similar cells of the glands along the greater curvature contain few granules, but a great deal of prozymogen. These cells differ, as Langley (1882) pointed out, in secretory equilibrium.

It is conceivable that mucous cells similarly differ in secretory equilibrium, and that, while the ultimate product of their secretory activity may be the same substance, they contain the antecedent substances in varying proportions. Some such explanation as this must be resorted to, in order to explain why similar cells of the same tubule differ in their staining capacity.

The glands of Brunner of the rabbit are mixed glands. The bulk of the cells composing the tubules are mucous cells similar in all important respects to those in other mammals. In many cases the dark cells forming the terminal acini or tubules are specifically different from the mucous cells. There are no intermediate stages, and these cells do not under any conditions contain mucin or its stainable antecedents. They contain zymogen granules, easily visible (as Schwalbe first pointed out) in the fresh cell, and prozymogen. By the microchemical reactions for iron and phosphorus these elements may be demonstrated to be fundamentally different from the contents of the mucous cells.

As regards the similarity of the glands of Brunner to the pyloric glands of the stomach, it may be said that in nearly all cases examined slight differences in structure could be discerned. I do not, however, regard these differences as of fundamental importance. Certainly they are primitively cells of the same type. An interesting fact is that the greatest differences between the cells of the two sorts of glands were found, in the series of animals studied by the writer, in those animals in which the stomach was highly specialized. This fact can be explained by the assumption that the two groups of glands were primitively similar, and that their great dissimilarity in the case of animals with specialized stomachs is due to the fact that the pyloric glands have also been modified in the course of this specialization. The writer, however, hesitates to generalize in this respect until a much larger series of animals has been studied by the methods employed in this research.

The question of the phylogeny of the glands of Brunner is an exceedingly difficult one to discuss. Up to the present the only clear-cut theory of their origin advanced is that of Oppel (1897). This author expresses his views as follows:

Bei zahlreichen niederen Wirbeltieren finden sich Spuren einer Tendenz der Pylorusdrüsen, sich über den Sphinkter hinaus auszubreiten, so z. B. bei Urodelen, wo eine scharfe Grenze zwischen den letzten Pylorusdrüsen und den Darmdrüsen überhaupt schwer zu ziehen ist. Die letzten Pylorusdrüsen zeigen ferner bei manchen Reptilien und Vögeln an ihren unteren Enden die Tendenz, sich stärker zu entwickeln, eine Tendenz, die auch noch bei Säugern zum Ausdruck kommt. Verbinden wir beides, so werden wir leicht den Vorgang der Entstehung der Brunnerschen Drüsen so deuten können, dass die Drüsen der Pylorusdrüsenzzone über den Sphinkter hinauswachsend und zu einer excessiven Entwicklung gelangend, die Muscularis mucosae durchbrechen und so zu Brunnerschen Drüsen werden.

The attractiveness of this hypothesis becomes apparent when we examine such a case as that of the opossum, in which the glands all open on circumscribed areas of the intestine, covered by gastric epithelium. The facts in favor of the hypothesis are briefly: the contiguity of the glands of Brunner to the pyloric glands in the less specialized mammals; and the great similarity of the cellular components. As regards the similarity of the cells, however, it must be remembered that the cells of the glands of Brunner resemble just as strongly the mucous cells of many buccal, oesophageal, and tracheal glands. We are thus reduced to the fact of contiguity as an effective argument for the phylogenetic development of the pyloric glands into glands of Brunner. Furthermore, in accepting this hypothesis we must assume that the epithelium of the small intestine is specifically differentiated from that of the stomach, not only in the adult, but in the embryo at the time the glands of Brunner are formed; that is to say that at a time when no structural differences can be discerned between the cells of the gastric and intestinal epithelium the cells actually have a different developmental potential, those of the stomach having lost the power of developing into cells of the intestinal type and those of the intestine that of developing into cells of the gastric type. We must also assume that there is a mingling of this gastric hypoblast and intestinal hypoblast in the region of the formation of the glands of Brunner, because

ontogenetically the glands of Brunner do not develop as downgrowths of pyloric glands, but develop simultaneously as independent elements in the duodenum. Furthermore, if we assume this extremely early specification of the respective epithelial elements of the stomach and intestine, how are we to explain the occurrence of glands of Brunner in the horse at a point seven meters from the pylorus, and the relatively great extent of the glands in the rabbit, sheep, pig, and man? It might, of course, be urged that it is possible that in the rapid growth of the midgut, gastric epithelial elements may be carried a considerable distance from the pylorus and there serve as foci for the development of glands of Brunner. A similar argument might be employed to explain the occurrence of characteristic intestinal epithelium in the stomach, as observed by Schaffer (1897), Boeckelman (1902), and Hári (1901). Such arguments are unanswerable because they do not admit of proof or disproof.

It does not seem possible to me to reconcile the facts of the distribution of the glands of Brunner and of their ontogenetic development with Oppel's theory that they are developed as a further downward growth of the pyloric glands into the intestine. For the present it would seem to be more probable that the glands of Brunner are cænogenetic structures developed in mammals from the hypoblast of the midgut. The occurrence of serous tubules in the glands of Brunner of the rabbit is evidence of a new functional need in the intestine.

Oppel (1899) has, however, promised further explanations of his theory to adapt it to the facts of distribution, and in the meantime these may be awaited with interest.

In conclusion I should like to emphasize the fact that I do not regard the evidence brought forward in this investigation to show that the glands of Brunner are mucous glands as at all excluding the possibility that they also form small quantities of digestive ferments. The latter, however, if they are formed, are not present in sufficient quantities to appear in the cells as definite formed elements recognizable by the microscopic or microchemical means at our disposal.

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## EXPLANATION OF FIGURES

For the drawings, of which Figs. 1-11 and Fig. 13 are reproductions the author is indebted to the skill of Mr. Leonard H. Wilder. All drawings are from camera lucida outlines.

## PLATE XIX

FIG. 1.—Section including a portion of the pars pylorica ventriculi and the upper end of the duodenum of the opossum, showing the peculiar defect of the tunica mucosa on which the glands of Brunner open.  $\times 40$ .

## PLATE XX

FIG. 2.—Transverse section of two tubules of the glands of Brunner of the opossum. Ferric alum hæmatoxylin. The drawing shows the structure of the cells composing these tubules in the condition of incomplete loading. The secretion is subdivided into a proximal and distal mass by a band of cytoplasm. Some basal cytoplasm still remains at the attached end of the cell and around the nucleus.  $\times 1100$ .

FIG. 3.—Transverse section of a tubule of the glands of Brunner of the opossum in the same physiological condition as those in Fig. 2, stained with stronger muchæmatein. The granular character of the secretion, its subdivision into two masses, and the clustering of the granules of the proximal mass around the oval or flattened nucleus are well shown.  $\times 1100$ .

FIG. 4.—Tubule of the gland of Brunner of the opossum in the condition of maximum loading; iron hæmatoxylin. The two masses of secretion are not to be recognized, the transverse band of cytoplasm separating them having disappeared; basal cytoplasm less than in Fig. 2; nucleus more flattened.  $\times 1100$ .

## PLATE XXI

FIG. 5.—Portion of a lobule of Brunner's glands of the rabbit, stained in iron hæmatoxylin. The figure shows nine mucous tubules and one serous acinus in transverse section. In the former the subdivision of the secretion into two masses is indicated in most of the cells.  $\times 500$ .

FIG. 6.—The serous acinus shown in Fig. 5 more highly magnified. In each cell the outer zone filled with deeply staining prozymogen and the inner clear zone containing a few granules of zymogen are shown. Most of the zymogen granules which are present in the living cell have been lost in fixation.  $\times 1300$ .

FIG. 7.—Fresh serous acinus from the rabbit's glands of Brunner showing inner zone filled with zymogen granules.  $\times 1300$ .

FIG. 8.—A tubule of Brunner's glands of the opossum. The section has been treated with water and then stained with stronger muchæmatein. The granules visible in Fig. 3 have given place to a deeply stained coarse-meshed network.  $\times 1100$ .

## PLATE XXII

FIG. 9.—Tubule of the glands of Brunner of the muskrat. Composed of large mucous cells with flattened nuclei. Transverse band of cytoplasm separating the contained secretion into two masses is readily visible.  $\times 720$ .

FIG. 10.—Portions of three pyloric glands of the muskrat. Note the smaller cells, larger nuclei, and more abundant cytoplasm as compared with Fig. 9.  $\times 720$ .

FIG. 11.—A small lobule from the glands of Brunner of man.  $\times 190$ .

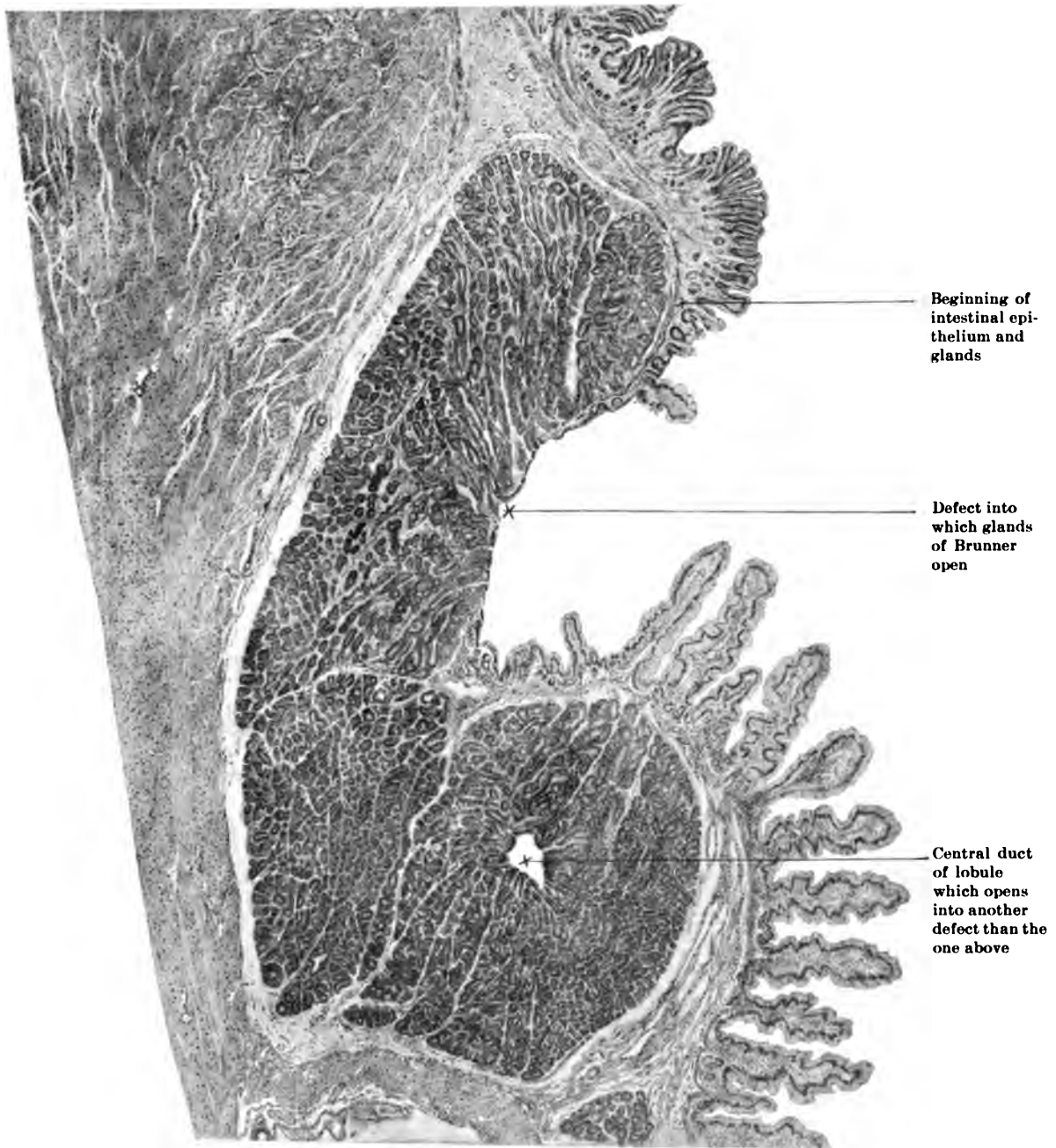
FIG. 12.—A portion of a lobule of the glands of Brunner of man located in the tunica mucosa. The duct of the gland opens into a gland of Lieberkühn, where a sudden change in character of the cells takes place. The cells of the duct become richer in secretion the farther they are from the point of entrance into the intestinal gland, the nuclei at the same time becoming more flattened. In many of the cells may be seen the transverse band of cytoplasm separating the secretion into two masses.  $\times 280$ .

## PLATE XXIII

FIG. 13.—Section of the duodenum of man. The superficial epithelium and portions of the villi in this section were unfortunately lost. The grouping of the glands of Brunner in the tunica mucosa and tela submucosa are shown, as well as their opening into the glandulæ intestinales.  $\times 66$ .

## PLATE XXIV

FIGS. 14, 15.—Photomicrographs of section of the duodenum of man stained in stronger muchæmatein. These figures show well the topography of the glandular lobules as well as the intensity of the stain which their contained mucin takes in this solution.  $\times 37$ .



L. H. WILDER

FIG. 1



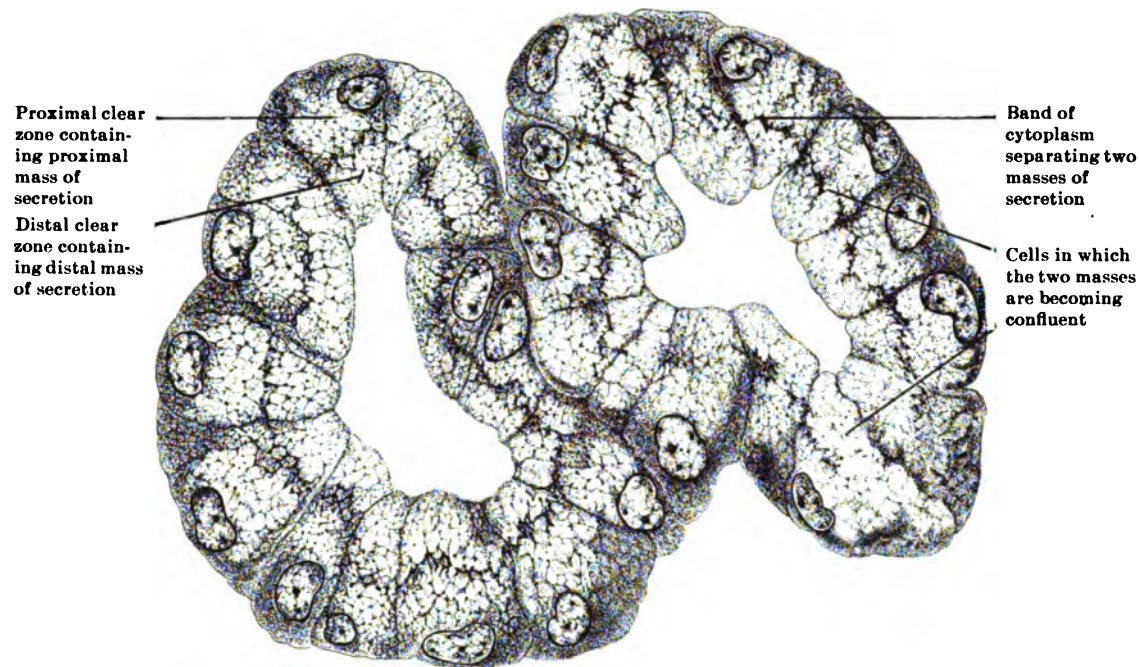
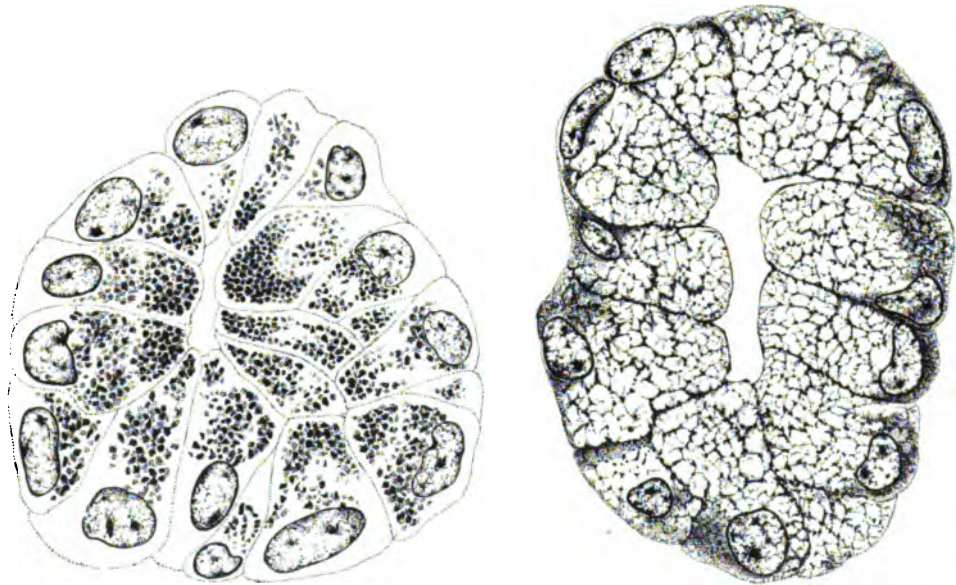


FIG. 2



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FIG. 3

FIG. 4



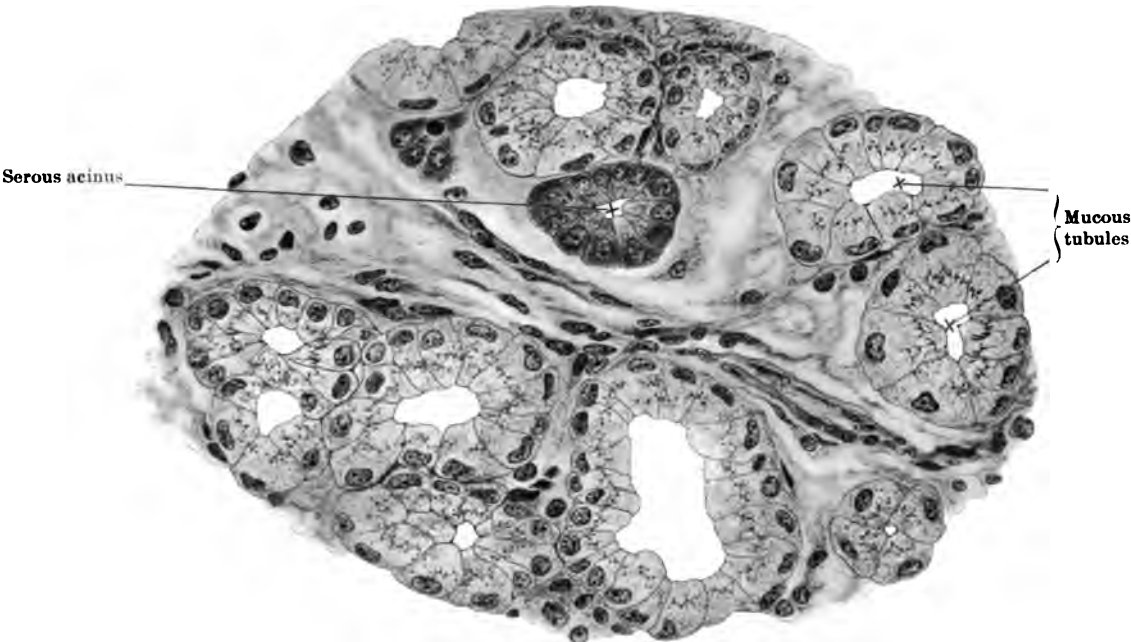


FIG. 5

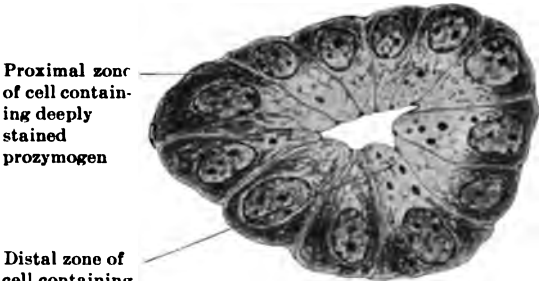


FIG. 6

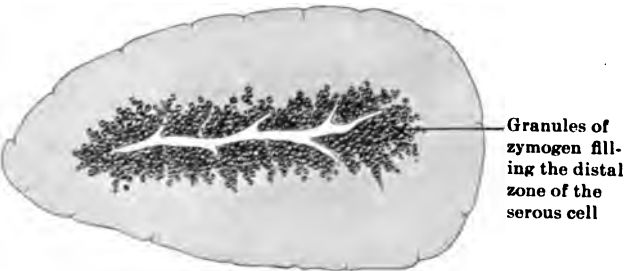


FIG. 7

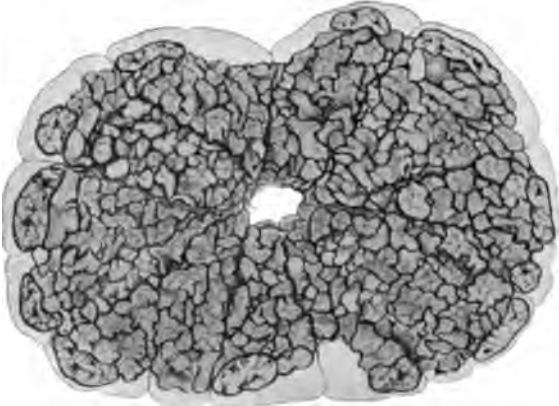


FIG. 8

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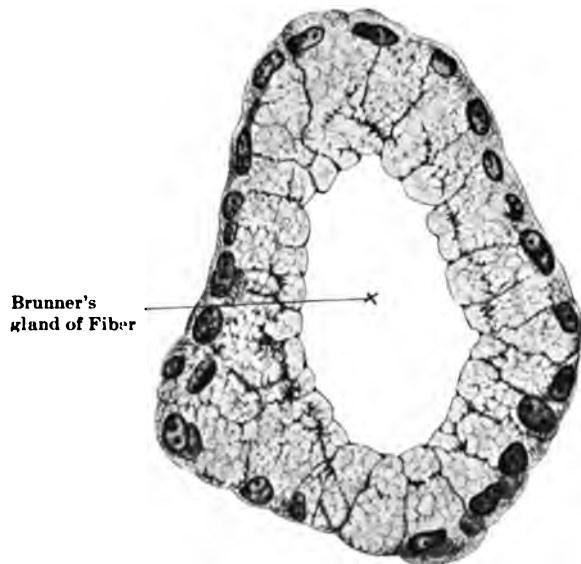


FIG. 9

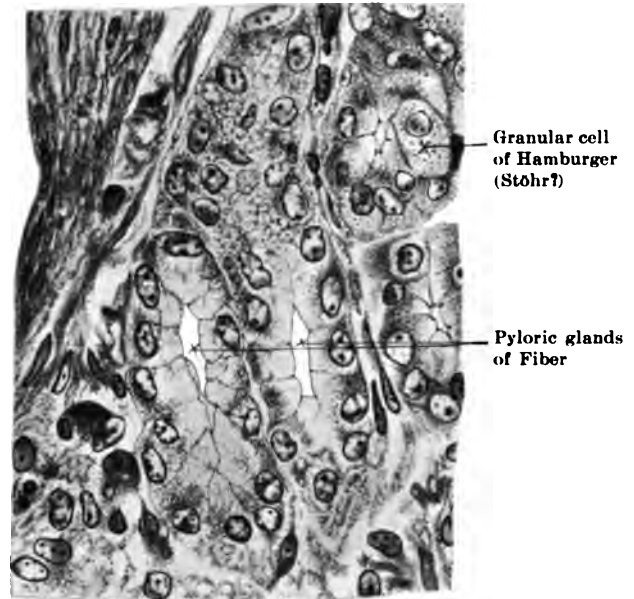


FIG. 10

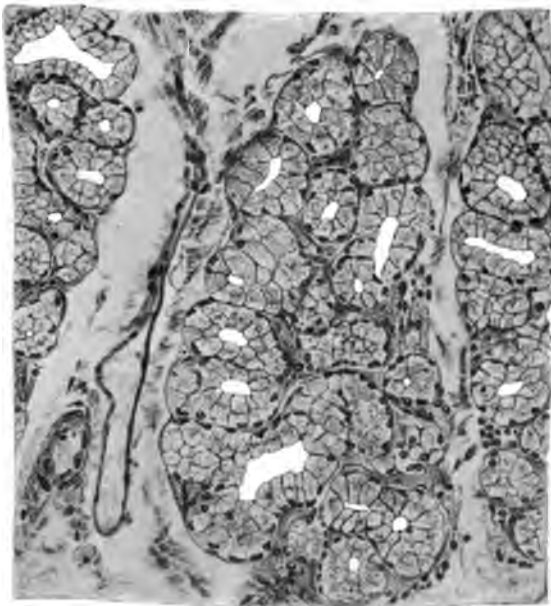


FIG. 11

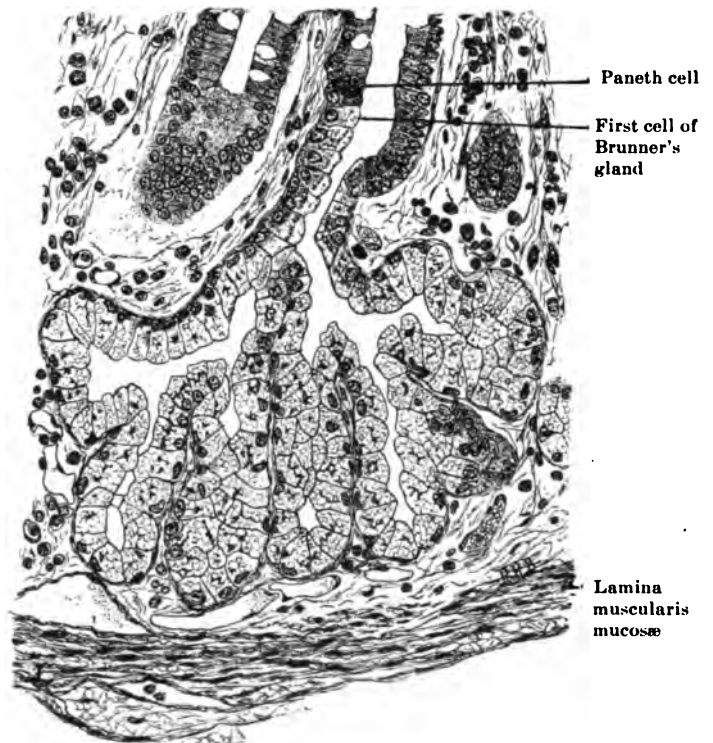
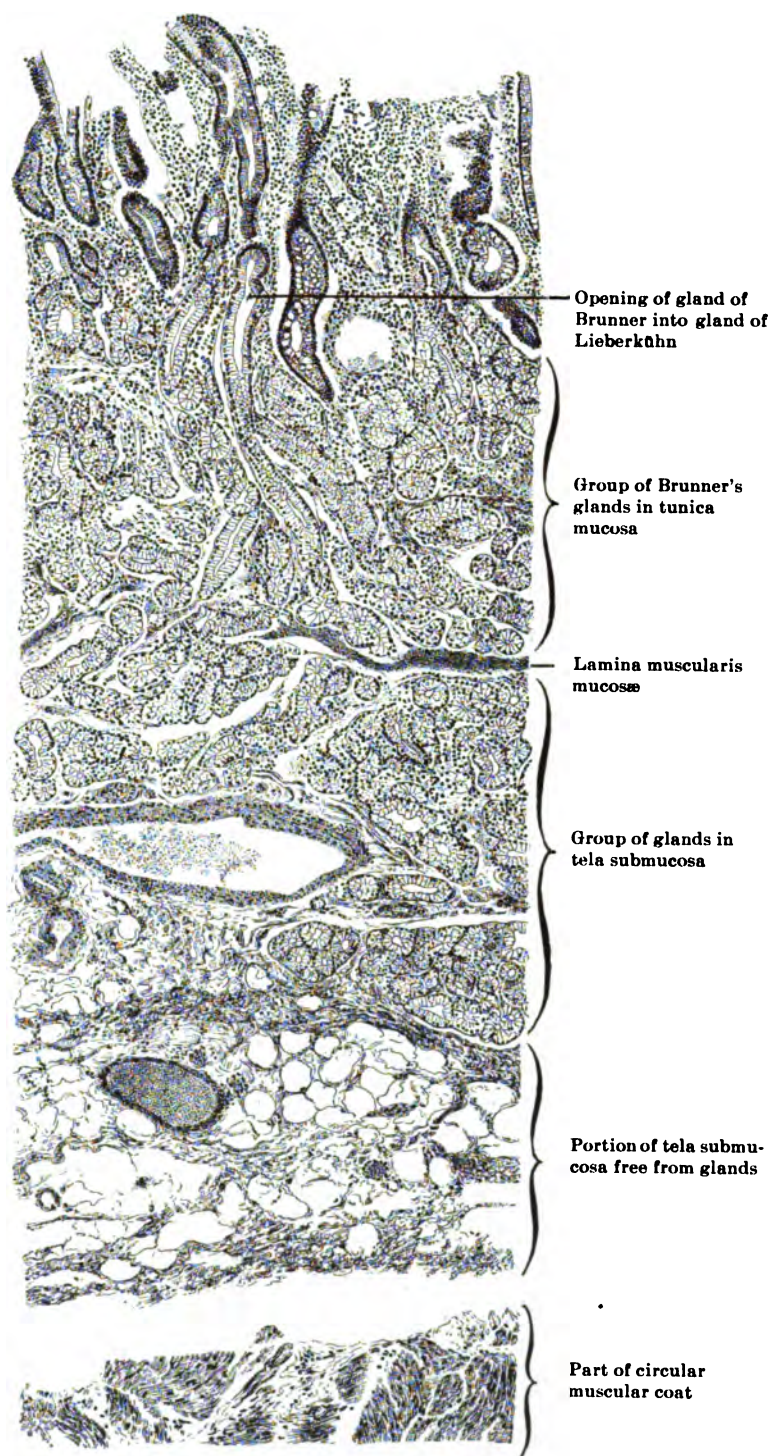


FIG. 12





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FIG. 13



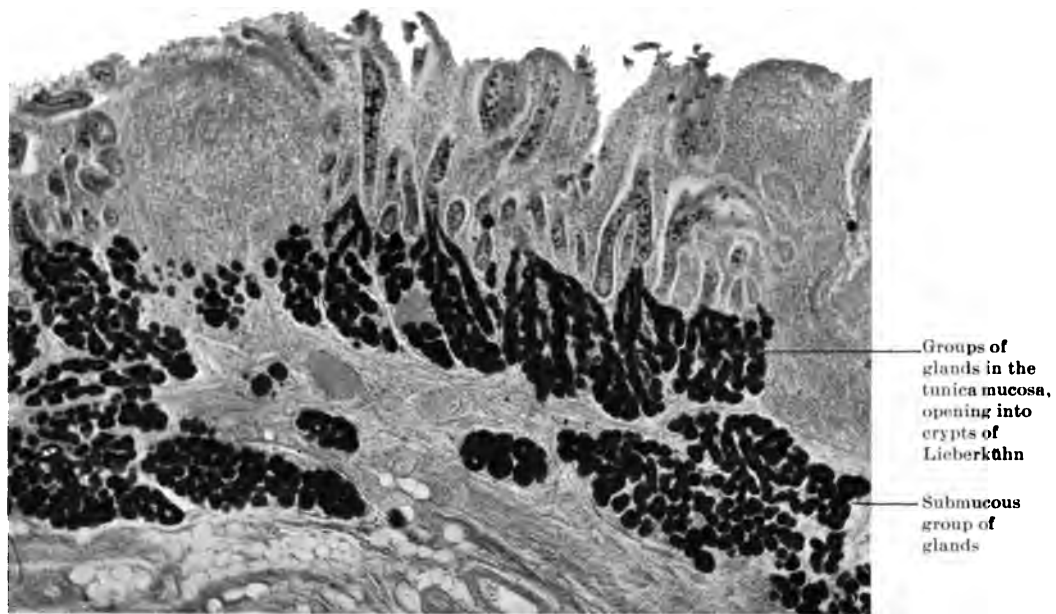
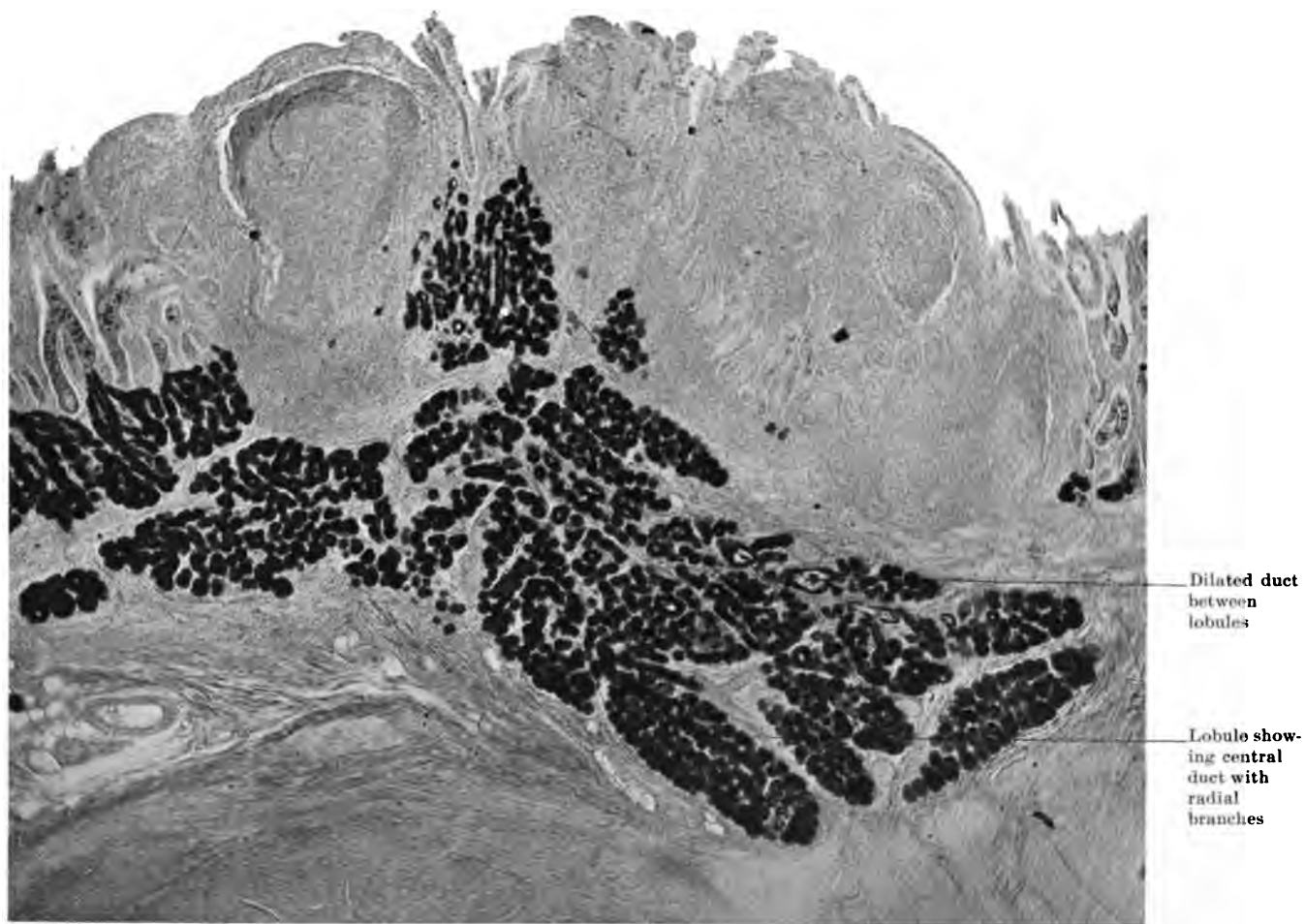


FIG. 14





## **MITOSIS IN PELLIA**





## MITOSIS IN PELLIA

CHARLES J. CHAMBERLAIN

JUST as an understanding of the gametophytes of the flowering plants has been gained by a comparative study of the progressive reduction of the gametophytes of the higher cryptogams, so, in our opinion, the processes of nuclear and cell division in the flowering plants will be understood only after an investigation of these processes in lower forms; and just as the Pteridophytes show the transitions which have cleared up the homologies of the gametophytes, so, it seems probable, the Hepaticæ, in their modes of mitosis, show the transitions which will lead to a correct interpretation of mitotic phenomena in the flowering plants. The Hepaticæ, however, have received comparatively little attention from cytologists.

In 1893, Schottlander (30)<sup>1</sup> described the sexual cells of several liverworts, paying particular attention to spermatogenesis. In the antheridia of *Marchantia* he found that the centrosomes divide during the anaphases of mitosis, so that each daughter-nucleus is accompanied by two centrosomes; but in the egg, centrosomes were not identified positively.

In 1894 Farmer (8) reported a quadripolar spindle in spore mother-cell of *Pallavicinia*. According to this account, the chromatin of the spore mother-cell breaks up into sixteen chromosomes, four of which are then conveyed simultaneously to each of the four spores. In the same year Farmer (9), in collaboration with Reeves, described centrospheres in the germinating spores of *Pellia*. In the following year Farmer (10) published a more extended paper dealing with spore-formation and nuclear division in *Fossombronia*, *Pellia*, *Conocephalus*, and several other forms. In most of these forms, centrosomes were observed to play an important rôle in mitosis. The occurrence of centrosomes in *Pellia* was confirmed by Strasburger (33) from Farmer's own preparations.

In 1899 Davis (6) studied the spore mother-cell of *Anthoceros*. In the two divisions by which the tetrad is formed from the mother-cell, the spindle, during the metaphase, is flattened at the poles and entirely lacking bodies which might be interpreted as centrosomes or centrospheres. The spore walls are described as being formed independently of the spindle.

The following year Van Hook (38), with more favorable material, confirmed Davis's statement that there are no centrosomes in the spore mother-cells of *Anthoceros*, but found the spindle functioning as in *Lilium* in the formation of spore walls. In the same paper Van Hook figured and described definite centrosomes in the vegetative cells of the gametophyte of *Marchantia*.

<sup>1</sup> Figures in parentheses refer to literature cited at the end of the article.

In 1901 Davis (7) made a detailed study of mitosis in various phases of the life-history of *Pellia*. Centrospheres were found during the early divisions in the germinating spore, but could not be identified in the sporophyte or in later stages of the development of the gametophyte.

At this time it hardly seems desirable to make a more extended résumé of the literature, since it is still too incomplete and indefinite to warrant generalizations. In presenting our own results, we shall occasionally refer to the preceding papers and also to papers dealing with mitosis in other groups.

#### MATERIAL AND METHODS

Most of the material for this work was collected near Bonn in Melbthal and in the Siebengebirge. Early in October the spore mother-cells of *Pellia* are already quite deeply lobed, and occasionally a sporogonium is found in which the spores are already formed. By the middle of November nearly all of the spore mother-cells have divided and many of the spores have germinated. The winter of 1901-2, in the Rhine Province, was a very mild one, and germination proceeded with only occasional interruption throughout the entire season. Material brought into the laboratory at any time after the middle of November developed much more rapidly than in the open, and would shed the spores within a week or ten days.

Before placing the material in the fixing agents, the calyptra was dissected away and about one-third of the sporogonium cut off with a razor, thus freely exposing the spores. In a few cases the mass of spores, held together only by the elaters, was removed from the sporogonium, but while not nearly so many spores were lost as might be anticipated, this tedious method was found to be unnecessary, since the other process readily yielded smooth sections as thin as  $2\mu$  or  $3\mu$ .

Several fixing agents were used, but only two gave thoroughly satisfactory results. These were chromo-acetic acid (0.8g. chromic acid, 0.5c.c. glacial acetic acid, 100c.c. water) and a modification of Flemming's solution (0.5g. chromic acid, 0.5g. glacial acetic acid, 1 per cent. osmic acid 10c.c., water 100c.c.). While achromatic structures stain more readily after solutions containing some osmic acid, equally good preparations were often obtained from material fixed in the former solution.

Most of the sections were cut at  $2\mu$  or  $3\mu$ , but sections  $5\mu$ , and even  $10\mu$  or  $15\mu$ , in thickness were used in determining the number of asters and in counting chromosomes.

Haidenhain's iron alum hæmatoxylin, with or without a slight tinge of erythrosin, Congo red, or orange G, gave fairly satisfactory preparations, but gentian violet proved to be so much superior in differentiating kinoplasmic structures that safranin and gentian violet, sometimes with the addition of orange G, were used in most of the work. Sections were stained, usually over night, in safranin (1g. safranin in 100 c.c. of 50 per cent. alcohol), then washed in 50 per cent. alcohol until all red color was removed from the achromatic structures, and then stained for one or two hours in gentian violet

(saturated aqueous solution). From the gentian violet the preparations were transferred directly to absolute alcohol, where they were quickly dehydrated, the process being hastened by moving the slide to and fro in the alcohol. Clove oil was used for clearing. The clove oil should be rinsed off with good cedar oil, otherwise the gentian violet gradually fades. When orange G was used the preparations were taken from the gentian violet, dipped a few times in water, stained for one minute in orange, and then transferred to the absolute alcohol.

In studying the preparations hollow glass globes, filled with various solutions, served as ray filters and condensers. A light blue solution of ammonia copper sulphate was used for most of the work, but occasionally a light violet solution of permanganate of potash, imitating the gentian violet stain, gave a sharper differentiation of the kinoplasmic structures.

While the work deals chiefly with the first three divisions of the germinating spore of *Pellia epiphylla*, and in these divisions is largely confined to the centrospheres, asters, and spindle, mitosis was studied in other phases of the life-history of this genus, and also in several other liverworts, among which were *Conocephalus*, *Marchantia*, *Aneura*, *Pallavicinia*, *Scapania*, *Lophocolea*, and *Porella*.

The principal results of the investigation were presented in a *Vortrag* before Professor Strasburger and the advanced students of the Bonn laboratory in February, 1902, and in July of the same year a brief résumé was presented before the botanical section of the American Association for the Advancement of Science.

#### THE SPORE MOTHER-CELL

The spore mother-cell was observed in *Pellia epiphylla*, *P. calycina*, *Aneura multifida*, and in *Porella platyphylla*. In all of these forms the nucleus occupies a central position during the development of the lobes which are to become spores. It seems probable that the nucleus is concerned in the formation of the lobes. We found nothing to support Davis's (7) statement that the nucleus lies in one of the lobes until shortly before the first division of the mother-cell. No quadripolar spindles, like that described by Farmer (7) for *Pallavicinia*, were found in any of the above-mentioned forms. On the contrary, the four spores in all these cases are formed by two successive divisions, as described by Farmer (10) and by Davis (7) for *Pellia epiphylla*. Unfortunately, no material of *Pallavicinia* in this stage was available, but the striking resemblance of Farmer's (8) figures to the mitoses in deeply lobed mother-cells of other Jungermanniales leads us to suggest, as Davis (7) has already done, that Farmer (8) may have misinterpreted the quadripolar figure in this genus.

#### THE GERMINATING SPORE

The first, second, and third mitoses in the germinating spore of *Pellia* cannot be regarded as distinct types, for with diligent searching one could select a series of mitoses at the second division, or even at the third, which would be identical with a

series at the first division. In fact, we have used Figs. 7, 8, and 19 of the third mitosis to illustrate also the same stages in the first and second mitoses. Nevertheless, it is true that, in a great majority of cases, kinoplasmic activity is most energetic during the first division, and that in succeeding divisions it becomes less and less conspicuous until centrospheres and asters cease to attract any attention, and it finally becomes doubtful whether they are present.

#### THE FIRST MITOSIS IN THE GERMINATING SPORE

As the nucleus of the germinating spore increases in size preparatory to the first division, the area immediately surrounding it becomes comparatively free from starch grains and coarser granules (Plate XXV, Fig. 1). It seems reasonable to suggest that some substance, escaping from the nucleus into the cytoplasm, causes this zone and acts as a stimulus to the formation of the extra-nuclear portions of the achromatic figure. It is not impossible that such a substance might actually take the form of a centrosphere. (The origin of the aster will be considered when dealing with the second division.) After the spirem has become segmented into chromosomes the nucleus elongates and the nucleolus appears very much vacuolated (Fig. 2). At this stage a pair of dome-shaped caps (Figs. 3, 4) may be recognized at opposite poles of the nucleus. These caps, which will be considered later, appear in transverse section as a delicate ring, but a similar section of the completed spindle shows a dense mass of fibers (Fig. 5).

During the earlier prophases the poles of the spindle are usually rounded (Figs. 3, 4, 6), but, as the metaphase approaches, the caps (Figs. 3, 4) which have given the poles of the spindle a rounded form become resolved into fibers, and the poles may vary in shape from sharply pointed figures, like that shown in Fig. 10, to such broad, indefinite ones as those shown in Fig. 8 (Plate XXV) and Fig. 27 (Plate XXVII). Spindles with three and even more poles are not very rare. They do not originate like the multipolar spindles of the spore mother-cells of vascular plants, but are preceded by the bipolar condition or are formed through the influence of three or more centrospheres or asters (Plate XXVI, Fig. 16, Plate XXVII, Fig. 23). During the anaphases the poles of the spindle are sometimes sharp and sometimes indefinite.

In the prophases it is plain that the achromatic figure is made up of the asters and two half-spindles (Fig. 6). As the spindle continues to develop, some of the fibers—the mantle fibers—become attached to the chromosomes; the other fibers increase in length until they reach the opposite pole, thus forming a part of the central spindle.

While the poles are separating from each other, radiations are easily seen, and they continue to be fairly conspicuous until the spindle has reached its full length, when they rapidly disappear, losing their staining capacity first at the peripheral ends, then throughout their entire length, and finally becoming indistinguishable. When the metaphase is reached, the radiations have usually disappeared (Fig. 7), and during the anaphases, while the chromosomes are passing to the poles, it is very seldom that any trace of radiations can be found. In the telophases, however, the radiations

reappear, but are not centered in any single point. When the nuclear membrane begins to form, the radiations again become indistinct and disappear as before. Just before the spindle reaches its full length (Fig. 6), the radiations often attain their greatest prominence, sometimes appearing as extremely coarse strands. In nearly all cases, even in very thin sections, some of the rays can be traced from the pole of the spindle to the *Hautschicht*. The diameter of the rays is usually greater at the polar end, but a slight increase in diameter at the *Hautschicht* also is not uncommon. The rays are usually simple, but may be branched especially during the earlier stages.

It is worthy of note that the radiations are most pronounced and stain most deeply with gentian violet, while the nucleus is elongating and its poles are separating from each other; and, further, that during this period many of the radiations connect the poles with the *Hautschicht*. The explanation which we venture to suggest is that the radiations take an active part in separating the poles from each other. The fact that the radiations disappear as soon as the poles have reached their widest separation supports this hypothesis. The reappearance of the rays in the telophase does not seem to be so definitely concerned with movement, because they again disappear before the nucleus has perceptibly changed its position: still, it is possible that there may be a slight movement of the nucleus toward the center of the new cell. The reappearance, however, takes place as the nuclear membrane begins to be formed, and it may be an expression of kinoplasmic activity during the formation of a *Hautschicht* surrounding the nuclear membrane, or the rays may be contributing to the formation of the nuclear membrane itself, which, we believe, is largely kinoplasmic in its nature.

#### THE SECOND MITOSIS IN THE GERMINATING SPORE, WITH REMARKS ON APICAL CELL, ANTHERIDIA, NUCLEOLI, AND CHROMOSOMES

The second mitosis is remarkably easy to fix and stain; so that, while the first mitosis, if equally well prepared, might show the early prophases with a little more clearness, our material afforded a better study of these stages during the second mitosis.

In studying the second mitosis, special attention was devoted to the centrosphere and to the origin of the achromatic structures. The terms "centrosome" and "centrosphere" are frequently confused. Until much more is known about the origin of these structures and their relation to each other, it is hardly worth while to attempt any definitions. A typical centrosphere—as the term is used in this paper—is shown in Fig. 12 (Plate XXVI). The centrosphere consists of the same substance as the astral rays and the spindle fibers. The elongated body toward which the rays converge in Fig. 15 is also a centrosphere, and the densely staining masses at the poles of the spindle in Fig. 6, although not organized into a definite body, consist of the same material as centrospheres and, at an earlier stage in mitosis, may have had a more definite form. We have not intended to represent a *centrosome* in any of our figures. Bodies which have the superficial aspect of centrosomes are shown in Figs. 14, 16, and 17, but here the sharply staining body at the center of the centrosphere is, without doubt, the cut end

of an astral ray. The structure at the upper pole in Fig. 9 certainly looks like a centrosphere containing a centrosome, but such an appearance is so rare that it seems safer to regard the sharply staining body as a chance granule. Still, it is evidently just such a body as this that Van Hook (38), in his recent study of *Marchantia*, interprets as a centrosome.

In the very early prophases a beautiful system of radiations becomes quite conspicuous. This system we regard as an aster, comparable with the asters of *Thallophytes* and of animals. The system first appears as a few fibers converging to a point which is usually in contact with the nuclear membrane or very near to it (Figs. 11-13), but, in some instances, may be at a considerable distance from the nucleus (Figs. 14-16). Persistent search failed to reveal any body which could be identified positively as a centrosome or centrosphere before the appearance of the aster, and even after the appearance of the aster and centrosphere, no centrosome could be distinguished. Granules, like those shown in all the figures, were frequently found in contact with the nuclear membrane after the nucleus had begun to enlarge, and it is probable that some of the granules were centrospheres, although no method was found for identifying them before the appearance of the rays. Bodies which bear remarkable resemblance to centrosomes (Figs. 14, 16, 17) and which, for a time, were interpreted as genuine centrosomes, proved to be merely the cut ends of coarse fibers. Sometimes several deeply staining points may be seen; such an appearance might easily be mistaken for a centrosphere containing several granules. In cases like those shown in Figs. 14-17, the "granules" are, without doubt, nothing but the cut ends of fibers. The two centrospheres in Fig. 17 are practically alike, but the one at the upper pole is represented in median section and the other in surface view, the fibers in vertical view appearing as dots. However, it must be admitted, that occasionally the deeply staining points are really granules (Fig. 9), but the cases are so rare that we have not regarded such granules as a functional part of the mitotic mechanism.

After a study of the germinating spore had failed to show any centrosomes, the nuclear figures were examined in other phases of the life-history, particularly in the apical cell and its younger segments, and in the developing antheridia. The apical cell and the rapidly dividing cells near it are quite favorable for study. The character of the mitoses in this region is represented in Figs. 9 and 10. The lower pole in Fig. 9 shows the more usual condition, although the rays are frequently as strongly developed as those shown at the upper pole, a considerable number of the rays reaching to the *Hautschicht*. A careful examination of this figure will show that there is no definite centrosphere like those in Figs. 12 and 13. In later stages (Fig. 10) the spindle becomes sharply bipolar and the radiations disappear.

The antheridia were examined with particular interest because Schottländer (30) had reported centrosomes during all stages in the development of the antheridium of *Marchantia*, and Belajeff (2) had found blepharoplasts throughout the development of the spermatogenous cells of *Marsilea*. However, nothing which could be interpreted

as a centrosome was found in our material, which furnished a series from the initial cell up to stages in which more than thirty cells appear in a transverse section of the antheridium. Unfortunately, the material showing the last two or three divisions preceding the formation of the spermatozoid mother-cells was not satisfactory, and, consequently, no positive statement can be made in regard to blepharoplasts, although we should assume them to be present during the last one or two mitoses.

In the germinating spore a differentiated area, already described as a centrosphere by Farmer (9), Strasburger (33), and by Davis (7), is often found at the center of the aster. The origin and behavior of this structure, which we regard as a genuine centrosphere, are rather puzzling. While we assumed that it must appear earlier than the rays, and that the rays were developed from it, the failure to identify the structure before the appearance of the rays, and its frequent absence when it might be expected to be present, led to a careful study of the subject. The conclusion was reached that the centrosphere gives rise to the rays, but that the rays may also contribute materially to the substance of the centrosphere.

Although we have not been able to make any satisfactory study of living material, we believe that appearances warrant the theory that there is a streaming movement in the rays. Such a theory is not entirely new to zoologists. If the theory be true, when the streaming is toward the nucleus the centrosphere would increase in size, while a continued streaming toward the periphery would cause the centrosphere to disappear. In regard to the origin of the rays, nothing more definite was determined. Finely granular areas, showing a tendency to stain with gentian violet, were sometimes seen in earlier stages, but the actual formation of rays or centrospheres from these areas could only be surmised. These areas do not seem to differ essentially from those which we (4) have already observed accompanying the male nuclei of *Pinus Laricio*. In some of Miss Ferguson's (13) figures of the same species and of *Pinus rigida* the areas approach the form of definite centrospheres. The aster appears so suddenly that its mode of development is largely conjectural. In a fully developed aster, there is usually an increase in the diameter of the ray at the centrosphere (Plate XXVI, Figs. 13 and 16), and occasionally a slight enlargement at the *Hautschicht*. An enlargement of the ends of the rays, as shown in Fig. 13, is just what should be expected if there is a streaming of material. The variability in the size of the rays and their irregularly granular character also favor the theory that they are lines of streaming material. The tendency of small nucleoli or microsomes to collect on the rays, as pointed out by Schaffner (27) in his study of *Lilium*, and as is familiar to all who have seen mitoses in the embryo-sac of *Lilium* and similar forms, is another argument in favor of this theory.

The asters arise at opposite poles of the nucleus, but not simultaneously. Serial sections of a large number of nuclei were examined before this conclusion was reached. We can hardly understand Davis's (7) statement that in his studies he "has never found a nucleus with a clearly defined solitary aster beside it. This is a very important point and the search was persistent." In our own preparations of the second and



third mitosis we never found anything but the solitary aster in the earliest stages. In studying this point, reconstructions were made from thin sections, and series were cut thick enough to include the entire nucleus. It is true that the first aster does not usually reach its fullest development before one appears at the opposite pole. In Figs. 14 and 15 and also in Fig. 19 (third division) there is only one aster. However, the second aster usually appears before development has proceeded so far. In spite of the fact that the two asters do not arise simultaneously, we can confidently support Davis's (7) conclusion that the two asters do not arise by the division of a single one. We found only two preparations in which the asters were less than  $180^\circ$  apart, except in case of tripolar figures, which were not very rare (Fig. 16, Plate XXVI—third pole not shown—and Plate XXVII, Fig. 23). In early stages the two poles usually differ from each other in appearance, one pole being rather pointed and the other comparatively blunt (Plate XXVII, Figs. 21, 22, 24, 25). Cases like Fig. 21 indicate that the blunt pole has been the last to develop. At this stage, neither pole is sharp, both being more or less rounded. The dome-shaped prominences or "caps," as they may be called, are by no means easy to interpret. In some cases the cap looks like a mere extrusion of the nuclear membrane, while in others the nuclear membrane is still intact after the caps have become quite conspicuous. The rounded ends indicate considerable pressure from beneath. That the cap is something more than a structure built up by fibers radiating from the aster is shown by its appearance and by the fact that in transverse section it presents a continuous line. The cap becomes finely granular and suggests a delicate membrane being resolved into fibers, rather than a membrane being formed from fibers (Fig. 4). In our opinion, the cap is derived from the outer portion of the nuclear membrane, or is itself a delicate layer—a sort of *Hautschicht*—immediately surrounding the nuclear membrane. The caps do not seem to be different from those seen in the root tips, as described by Némec (24), Schaffner (28), and others.

The rays of the aster do not penetrate the caps, but are closely applied to them. The aster exerts a strong pull, as may be seen during the period of elongation, although the elongation is due, in some degree, to pressure from within.

As in the first mitosis, the spindle in early stages consists of two half-spindles (Fig. 26). Until the caps become resolved into fibers they keep the spindle rounded (Fig. 26). The caps generally break up into fibers during the metaphase or early anaphases, and the poles of the spindle may then become blunt or irregular (Fig. 27). Occasionally the caps keep the poles of the spindle rounded even after rather late anaphases have been reached (Fig. 28).

The polar radiations generally disappear at the end of the prophase, are absent during the metaphase and anaphases, and reappear in the telophase (Figs. 26–29). That portion of the spindle which lies between the two caps is undoubtedly nuclear in origin. It consists of a very dense mass of spindle fibers which appear with remarkable suddenness (Fig. 20; cf. Fig. 5).

From observations on the nucleolus, we feel sure that this body contributes consid-

erable substance to the growing chromosomes. As the chromosomes increase in size, the nucleoli become more and more vacuolated, and material which resembles that of the nucleoli is often found adhering to the growing chromosomes. After the chromosomes have reached their full size, the nucleoli fragment, the fragments usually staining with gentian violet. Soon the entire nuclear cavity becomes filled with granular matter staining with gentian violet, and at this period the central portions of the spindle appear suddenly as the granular matter disappears. A few early spindles were noted in which this central portion did not seem to consist of sharply defined fibers. While such an appearance is often due to faulty methods, the sharply defined fibers in other figures in the same preparation favor the inference that these undifferentiated portions represent stages in the transformation of nucleolar matter into spindle fibers. In our opinion, these phenomena support Strasburger's (33) theory that the nucleolus contributes some of the material for the spindle.

Observations on the chromatin were merely incidental, but it is certainly safe to say that *Pellia*, in spite of the small size of its nuclei, is a favorable object for such study. As has just been mentioned, the nucleolus probably contributes something to the substance of the chromosomes. Although the chromosomes are small, they can be distinguished very early and seem to lose their identity much later than is usually the case. Mitoses in the venter of the archegonium show a longitudinal splitting of the chromosomes before the breaking down of the nuclear membrane, while in the germinating spores the splitting occurs much later.

The number of chromosomes in the gametophyte, as counted in the germinating spores and in the actively dividing region of the thallus, is eight. This number, however, is far from being constant. Both Farmer (10) and Davis (7) report occasional irregularities. In the present study, a few nuclei were found with only seven chromosomes, and nine chromosomes were counted in more than a dozen cases (Plate XXVI, Fig. 20). Long spindles upon which the chromosomes are irregularly arranged are not infrequent, and it seems probable that such a mitosis might result in an unequal distribution of the chromosomes, and thus account for variations from the typical number (Plate XXV, Fig. 8).

#### THE THIRD MITOSIS IN THE GERMINATING SPORE

While considerable attention was given to the third mitosis, an extended description is hardly necessary. Prominent asters (Plate XXVI, Fig. 19) like those of the two preceding mitoses are often present, but they are frequently absent, and the caps appear with only a few radiations (Fig. 18) or even none at all. There are no radiations in the metaphase (Plate XXV, Fig. 7). In short, it is possible to select from the third mitosis a series of stages identical with a typical series from the apical region of the thallus. At the fourth and succeeding mitoses the resemblance to the usual vegetative divisions becomes more and more pronounced, while asters and centrospheres become correspondingly rare.

## THE CENTROSOME PROBLEM

The centrosome<sup>2</sup> problem is one of extreme difficulty, and perhaps the difficulty is greater for the botanist than for the zoologist. At least, the difficulties are different in the two cases. That there are in animals well-defined centrosomes which function as organs of nuclear division, all investigators agree, and animals or tissues in which centrosomes do not occur are regarded as exceptions. The existence of the organ is not a serious problem; rather, the more recent investigations have sought to establish the permanent or transitory character of an organ which all admit to be present during mitosis. In plants, on the other hand, even the existence of a centrosome is a problem which must be considered separately for the different groups.

It is of interest to note that centrosomes in plants were first observed in diatoms in 1886 by H. L. Smith (31). When Guignard in 1891 published his classic paper on fertilization, botanists at once accepted the results and confirmatory accounts appeared. Strasburger (33) found centrosomes in *Larix*, Humphrey (18) in *Psilotum*, Mottier (22) in *Delphinium*, Schaffner (26) in *Alisma* and *Sagittaria*, Campbell (3) in *Equisetum*, Lauterborn (21) and Karsten (20) in diatoms, and other investigators reported centrosomes in various forms ranging from the algæ up to the flowering plants. In fact, the centrosome seemed to be as universally present in plants as in animals. Belajeff (1) and Farmer (11), however, failed to find centrosomes in *Lilium*. At the same time Strasburger (35), directing a remarkable group of investigators, attacked the problem in all the principal groups of plants. Those who studied Thallophytes found centrosomes, but those who studied Pteridophytes and Spermatophytes not only found no centrosomes, but, in tracing the origin of the multipolar spindle, they found conditions which seemed to preclude any such bodies. Just as the discovery of centrosomes was followed by confirmatory accounts, the multipolar spindle and the non-existence of centrosomes in the vascular plants received immediate confirmation. Guignard, Schaffner, and others still continued to find centrosomes in flowering plants, although these bodies, as represented in the figures, became noticeably less conspicuous than in earlier accounts. In Guignard's (15) recent studies of fertilization no centrosomes are represented in the figures, and no reference to any such structures is made in the text, even during the stage at which the famous "quadrille of the centers" was formerly (14) described. The fact that the great majority of cytologists, with the most approved technique and provided with apochromatic immersion lenses fail to find centrosomes in flowering plants, added to the fact that the mode of spindle-formation both in reproductive and in vegetative cells does not require the participation of a centrosome, makes the evidence overwhelming that the centrosome, as an organ of division, does not exist in this group.

In regard to the Pteridophytes, the evidence is similar, but not nearly so extensive. The blepharoplasts of Pteridophytes and Gymnosperms will be considered later.

<sup>2</sup>In referring to flowering plants no attempt has been made to distinguish between centrosomes and centrospheres. In describing mitosis in liverworts some writers have used these terms indiscriminately.

In the mosses the centrosome problem has received no serious attention, doubtless on account of the small size of their nuclei. Whether there is even a blepharoplast or not, still remains to be determined.

In the liverworts, no centrosome is found at any stage in the life-history. However, in *Pellia* and *Conocephalus*, and perhaps in all forms with such extensive intrasporal development of the gametophyte, a centrosphere appears during the early divisions in the germinating spore, but even in these few divisions the centrosphere is very transitory, not persisting from one nuclear division to the next, and appearing only irregularly during the division with which it is concerned. Still, this transitory centrosphere is a functional part of the mitotic figure during the first two or three divisions. In *Pellia*, at the fourth division, the centrosphere may or may not appear, and in subsequent divisions it was only rarely that we could identify the body at all.

Among the Thallophytes, sharply defined centrosomes have been described by competent observers who are thoroughly familiar with all phases of the centrosome problem.

In the fungi, judging from Harper's (16) work on various Ascomycetes, a centrosome is present during the period of free nuclear division in the ascus, when it functions in the formation of the spindle. After the period of free nuclear division, the centrosome behaves in a very peculiar manner in forming the young wall of the ascospore.

The centrosome has received more attention in the algæ than in the fungi. In papers by Farmer and Williams (12), and by Strasburger (34), centrosomes are described in the oogonia and segmenting eggs of *Fucus*.

During the early segmentations of the fertilized egg, Strasburger (34) was able to observe the division of the centrosome and to trace its continuity from one cell to another. In the development of the oogonium, however, no such continuity could be recognized. In the large apical cell of *Stypocaulon*, Swingle (37) found that the centrosome divides, giving rise to the two centrosomes from which the spindle is developed. He was able to recognize the centrosome even during the resting-stage of the nucleus. In the tetraspore mother-cell of *Dictyota*, Mottier (23) found comparatively large and somewhat elongated centrosomes. These bodies divide and, at least during divisions in the tetraspore mother-cell and in the early divisions of the germinating tetraspore, persist from one cell-generation to another. They develop asters and play an important part in the formation of the spindle.

Lauterborn (21) figures conspicuous centrosomes in *Surirella* and other diatoms. Karsten (20) also describes centrosomes in diatoms, and his beautiful preparations, which it was our pleasure to examine, show these bodies as sharply defined as in most animal mitoses. Both Lauterborn and Karsten agree that a centrosome, or at least a body derived from it, becomes cylindrical or ring-shaped, and functions as a spindle during mitosis. The centrosomes of diatoms stain intensely and are not surrounded by a centrosphere. Lauterborn found centrosomes even during the resting condition

of the nucleus and cell, but Karsten was not able to identify the body positively until the radiations began to appear. Davis (5) describes a centrosphere, but no centrosome, in the tetraspore mother-cell of *Corallina*. The centrospheres give rise to the spindle, and consequently play an essential part during nuclear and cell division. No centrospheres could be recognized during the resting-stage of the nucleus.

Thus it appears that in many of the algæ well-defined centrosomes are present, at least during certain phases of the life-history, and that the centrosomes may divide and persist from one cell-generation to another, while in other algæ the centrosome does not show such a degree of permanence. In the algæ which we have mentioned the centrosomes are not surrounded by a clear area. In *Corallina* it is to be noted that there is no centrosome, but only a centrosphere. In none of the algæ have centrosomes been traced throughout the life-history of the plant. In some fungi centrosomes are present during the mitoses concerned in the development of spores. Among the liverworts we doubt whether there is, at any period in the life-history, a centrosome like those described for the Thallophytes. The centrosphere appearing and functioning during only a few mitoses, has replaced the functional centrosome.

The polar radiations which are often conspicuous during mitosis in Pteridophytes, Gymnosperms, and Angiosperms, are of the same nature as those of Thallophytes and bryophytes, but in the higher groups (and, possibly, in most mitoses in the lower groups) a definite centrosome, or even a centrosphere is lacking. Centrosomes and centrospheres in vascular plants have been described and figured so frequently by such competent observers that he would be rash, indeed, who would claim that all such accounts have no foundation except in perverted imagination and preconceived theories. That theories suggested by the accounts of zoölogists and supported principally by misinterpretations of plant structures have caused exaggeration in the drawings and descriptions of botanists is probably true. While we believe that most of these centrosomes are to be interpreted as chance granules, nucleoli, pieces of chromosomes, etc., still we see no reason why a centrosome or centrosphere might not occur occasionally through atavism. The finely granular areas which have been noted during spermatogenesis in Coniferales and the similar areas which are often seen in Angiosperms, are, in our opinion, vestiges representing historically the centrosphere as it appears during the early mitoses in the germinating spore of *Pellia*.

The blepharoplasts described for various Pteridophytes and Gymnosperms are, in our opinion, to be interpreted as centrosomes. It seems to be true that in *Ginkgo* (17), *Cycas* (19), and *Zamia* (39) they appear only in the body cell and in the spermatozoids. In *Marsilea*, however, Shaw (29) traced them another cell-generation farther back, and in the same genus Belajeff (2) found blepharoplasts even during the earlier stages in the development of spermatogenous tissue. But, granting that the blepharoplast appears during only one or two cell-generations, this does not seem to be a valid argument against its centrosome character, for in *Pellia* the centrosphere is clearly distinguishable during only a few mitoses, and even in the multicellular Thallo-

phytes, if the centrosome should prove to be present throughout the life-history, it is at least much more conspicuous at some phases than at others. Plants furnish numerous illustrations of the gradual reduction, and even the disappearance of organs during phylogeny. Most botanists admit that in the earliest sporophytes all the cells were sporogenous; but, during phylogeny, portions of the sporogenous tissue became sterilized until the sporogenous tissue finally became much limited in extent and now appears only during a few cell-generations. During such reductions, functions of cells or organs may become completely changed, as in the case of the elaters of liverworts, which are, historically, sporogenous cells and often develop like sporogenous cells, even up to the spore mother-cell stage. In the formation of the ascospore, the function of the centrosome is not the same as during the mitotic divisions in the ascus. Other examples might be cited.

That the function of the blepharoplast is somewhat peculiar must be admitted. Radiations, however, and spindle fibers, which are often the most conspicuous accompaniments of centrosomes and centrospheres, are actively concerned in movement and are not essentially different from the radiations or cilia of blepharoplasts. In form and function centrosomes present so much diversity among themselves that the peculiarities of the blepharoplast need occasion no surprise. One has only to compare the typical spherical centrosome with the rod-like centrosome of *Dictyota*, the hollow cylindrical spindle of some diatoms, and with the centrosome which forms the *Hautschicht* of the ascospore.

We should conclude, therefore, that centrosomes, centrospheres, and blepharoplasts are historically related, and with their radiations, spindle fibers, and cilia are only different manifestations of kinoplasmic activity, movement in all cases being the principal function.

*Pellia*, with the prominent aster and centrosphere of its germinating spore becoming less and less distinct in succeeding mitoses until a condition is reached resembling that which prevails in the flowering plants, presents in its own life-history a great reduction of the aster and the disappearance of the centrosphere.

I am deeply indebted to Professor Strasburger for his kindly courtesy and helpful suggestions during my work in his laboratory.

#### SUMMARY

1. The principal part of the work deals with the first three divisions in the germinating spore of *Pellia epiphylla*. We have not intended to attack the excellent work of previous investigators, but rather have attempted to extend a little farther a knowledge of the phenomena of mitosis.

2. A centrosphere, but no centrosome, is very prominent during early prophase of the first mitosis in the germinating spore. The centrosphere is not present at all during the subsequent stages of mitosis. An aster is also conspicuous during the early prophase of the first mitosis, but disappears before the metaphase is reached.

Radiations reappear during telophases. The aster is believed to be concerned in separating the poles of the spindle; the radiations during the telophase may be concerned in forming the nuclear membrane or a *Hautschicht* about the nuclear membrane. In the second and third mitoses, the centrospheres and asters become more and more indistinct, and in succeeding mitoses the centrosphere becomes indistinguishable, and a few irregular rays replace the aster.

3. The rays are believed to be lines of streaming material, consisting of the same substance as the centrospheres.

4. Centrosomes, centrospheres, and blepharoplasts are believed to be the same structures historically, being only different manifestations of a common kinoplasmic activity.

5. No centrosomes or centrospheres were found during mitoses in the apical region of the thallus or in developing antheridia. It was not determined whether a blepharoplast occurs toward the close of spermatogenesis.

6. The central portion of the spindle is believed to be derived in large measure from the nucleolus.

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### EXPLANATION OF PLATES

All figures were made with a Bausch and Lomb camera lucida, Zeiss apochromatic immersion objective 2 mm. 1.30 N. A., and Zeiss compensating ocular 12; magnification, about 1,500 diameters. All figures are from *Pellia epiphylla*, Raddi, except Figs. 1, 2, 11, and 12, which are from *Pellia calycina*, Nees.

#### PLATE XXV

(Figs. 1-8, mitoses in germinating spore; 1-6, first mitosis, 7 and 8 third mitosis; Figs. 8 and 10, mitosis in apical region of thallus.)

FIG. 1.—Area about the elongating nucleus has become rather free from starch grains and larger granules. Asters and caps are present.

FIG. 2.—Peculiar aster at upper pole; the papilla indicates that it is pulling upon the nuclear membrane.

FIG. 3.—The cap is very conspicuous and the nuclear membrane is still intact.

FIG. 4.—A cap just beginning to break up into fibers. A transverse section at this stage shows a ring.

FIG. 5.—Transverse section of fully formed spindle.

FIG. 6.—Mitosis in late prophase; the spindle is evidently made up of two half-spindles, radiations conspicuous; definitely formed centrospheres are lacking.

FIG. 7.—Metaphase of third mitosis; no radiations or centrospheres are present. Figures of first mitosis are the same at this stage.

FIG. 8.—Irregular mitosis (third mitosis) in an unusually large spore, suggesting how nuclei with an irregular number of chromosomes might be formed.

FIG. 9.—Mitosis near apical cell; caps prominent and radiations reaching to the *Hautschicht*; at the upper pole is a granule resembling a centrosome.

FIG. 10.—Anaphase in mitosis near the apical cell; no asters or centrospheres are present.

#### PLATE XXVI

(Figs. 11-17, second mitosis; 18-20, third mitosis, in germinating spore.)

FIG. 11.—Very early prophase.

FIG. 12.—Centrosphere and radiations.

FIG. 13.—Very prominent centrosphere and radiations. The centripetal ends of the radiations have a pseudopodium-like aspect and suggest that the radiations are lines of streaming material.

FIG. 14.—Centrosphere in which the cut end of a fiber resembles a centrosome. There is no centrosphere or aster at the other pole.

FIG. 15.—Irregular, elongated centrosphere with prominent aster; no centrosphere or aster at the other pole.

FIG. 16.—Tripolar spindle, the third pole not shown. The cut end of a fiber resembles a centrosome. The pull upon the nucleus is evident; upper aster at some distance from the nucleus.

FIG. 17.—The two centrospheres are practically alike, but the upper one is shown in median section, while the lower one appears in surface view, the fibers having the appearance of granules within a centrosphere.

FIG. 18.—The more usual appearance of an early prophase at the third mitosis; prominent caps, but no centrospheres or very definite aster.

FIG. 19.—An exceptionally prominent centrosphere and aster at the third mitosis; no centrosphere or aster at the other pole.

FIG. 20.—Transverse section of mitotic figure at the third mitosis, just before the splitting of the chromosomes, showing nine chromosomes.

#### PLATE XXVII

(Figs. 21-9, second mitosis in the germinating spore.)

FIG. 21.—Cap more prominent at upper pole; nuclear membrane intact.

FIG. 22.—Nuclear membrane has broken down at the poles, but is still intact at the sides of the nucleus.

FIG. 23.—Tripolar figure.

FIGS. 24, 25.—Lower cap much broader than the upper; the granular matter within the nucleus is derived largely from the nucleolus and stains with gentian violet.

FIG. 26.—Late prophase; the achromatic figure evidently consists of two half-spindles.

FIG. 27.—Spindle very broad at the poles; a rather common form at this stage in the first three mitoses; no radiations or centrospheres.

FIG. 28.—The caps have kept the ends of the spindle rounded for an unusually long period; no centrospheres or radiations.

FIG. 29.—Telophase; radiations, but no centrospheres have reappeared.



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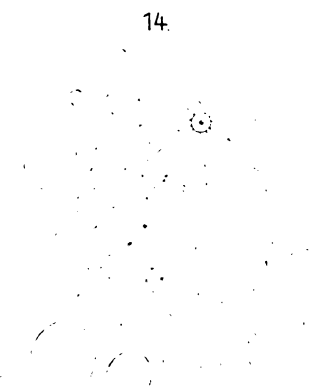
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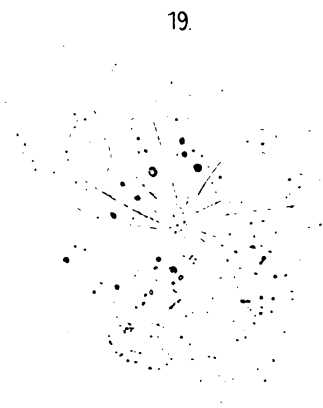
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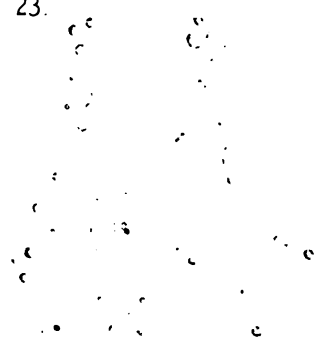
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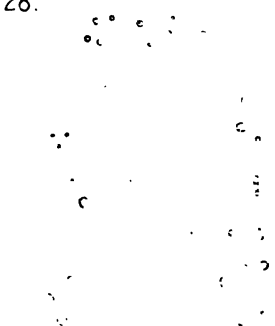
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**DESCRIPTION OF BRAIN AND SPINAL CORD  
IN HEREDITARY ATAXIA**



# A DESCRIPTION OF THE BRAINS AND SPINAL CORDS OF TWO BROTHERS DEAD OF HEREDITARY ATAXIA

CASES XVIII AND XX OF THE SERIES IN THE FAMILY DESCRIBED BY DR.  
SANGER BROWN. WITH A CLINICAL INTRODUCTION  
BY DR. SANGER BROWN

LEWELLYS F. BARKER

THE valuable material which forms the basis of this study was presented (for investigation) by Dr. Sanger Brown, of Chicago, to Dr. William H. Welch, Professor of Pathology in Johns Hopkins University, who kindly turned it over to me. I wish to express my thanks to both these gentlemen for the opportunity they have given me of studying the pathological changes in a disease so rarely met with.

The description of the anatomical findings will be preceded by a Clinical Introduction by Dr. Sanger Brown.

## CLINICAL INTRODUCTION BY DR. SANGER BROWN

As an introduction to Dr. Lewellys Barker's Anatomical Report, and for the purpose of facilitating correct deductions therefrom, as well as for the convenience of those interested who either have not read or do not now distinctly remember my clinical report of the series first published in 1892 in *Brain* and in the *North American Practitioner*, I shall undertake to supply some data intended to give a general idea of the characteristic clinical features, together with a reproduction of my original genealogical chart (see next page) showing the hereditary relation of the disease, and the age at which it first made its appearance in the individuals affected. Finally I shall furnish a more particular history of the cases supplying the material for Dr. Barker's report.

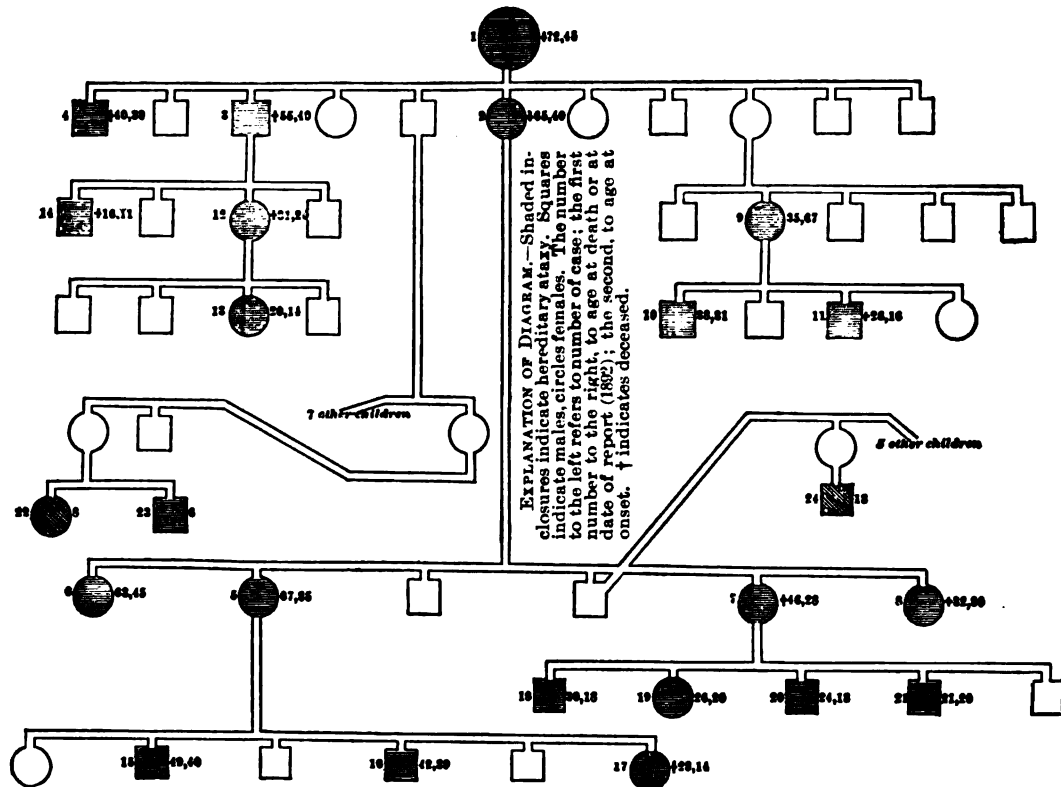
From a careful survey of all the cases comprised in the series, I presented in my original paper a general summary, which I here quote :

"Taking these cases alone for a text, and assuming them to be cases of hereditary ataxia, the following diagnostic criteria might be fairly deduced :

"Hereditary ataxy is a disease which may be traced through several—at least four—generations, increasing in extent and intensity as it descends, tending to occur earlier in life and to advance more rapidly. It usually attacks several members of the same family. It occurs most frequently between the ages of sixteen and thirty-five, but it may begin as early as eleven and as late as forty-five. It shows no marked preference for sex, but it descends through females four times as frequently as through males. Atavism rarely occurs. The influence of an exciting cause can rarely be

#### 4 DESCRIPTION OF BRAIN AND SPINAL CORD IN HEREDITARY ATAXIA

demonstrated, but in some instances a fall or injury has appeared to determine the onset; and any cause like child-bearing or lactation, which very much depresses the vital forces, may produce a rapid advance of all the symptoms. There is always considerable inco-ordination of all the voluntary muscles, and a sluggishness of the movements which they produce, when the disease is well established. This is usually noticed first in the muscles of the legs, but in a few months or years extends to the



FAMILY TREE OF HEREDITARY ATAXY

arms, face, eyes, head, and organs of speech. Sometimes it occurs first in the upper extremities, and sometimes in the organs of speech.

"The ataxy is often extreme, and the gait devious, the patient deviating several feet on either side of the intended line of progression before he loses the power of walking. The ataxy is not markedly increased by closing the eyes. The sense of posture is perfect.

"Some weakness of the muscles of the legs, without atrophy, is frequently an advanced symptom, and occasionally [in the late stages] there is permanent spastic contraction of the legs. In developed cases there are usually extensive choreiform movements of the head, and often of the arms, accompanying all voluntary move-

ments. These irregular movements occur in the hands, legs, or head whenever it is attempted to maintain either of these parts in a fixed position by a voluntary muscular effort. Movement ceases during sleep. The pulse-rate may be increased to 112 in advanced cases or may be normal.<sup>1</sup>

"There is usually some degree of static ptosis, with overaction of the levator on looking upward. In rare cases there may be temporary diplopia, in the early stages, due to weakness of the external [lateral] rectus. There is no nystagmus of any kind.

"Atrophy of the optic nerve is a constant and early symptom, and usually progresses slowly with the other symptoms. Rarely it begins earlier in one eye than the other.

"The response of the iris to light and accommodation is sluggish and diminishes with the advance of the optic-nerve atrophy; when this latter is complete, as may happen in advanced cases, there may be complete internal and external ophthalmoplegia.

"There is always marked disturbance of the articulation, probably due to incoordination of the muscles concerned, for weakness cannot be demonstrated. In some cases there is a troublesome tendency to strangulation in swallowing liquids, due to their getting into the larynx, but otherwise swallowing is in no way difficult.

"Occasionally the sphincters are slightly, but positively, affected, this symptom appearing only in those cases where spontaneous pains in the legs coexist, having some of the characteristics of those occurring in locomotor ataxy. Excepting the spontaneous pains already mentioned, there is no disturbance of sensibility. There are no vaso-motor or trophic symptoms, but there is a marked tendency to emaciation; there is no hypertrophy or valvular lesion of the heart.

"The knee-jerk is always exaggerated, and there is frequently ankle-clonus, and the cutaneous reflexes are also always exaggerated, but to a less degree. The exaggeration of the reflexes is an early symptom, and they often decline considerably when the disease is far advanced.

"There is never paralytic club-foot, nor any other deformity excepting rarely permanent spastic contractions of the legs in advanced cases. In none of these cases have the patients ever suffered from rheumatism, so far as I can learn.

"I wish to repeat that the above summary of the symptomatology of hereditary ataxy is only intended to apply to this particular series of cases; and I have only presented it in this way so that it might be the more easily compared with other series."

I shall now quote from my original paper the clinical notes of the cases upon which Dr. Barker's anatomical report is founded, indicating any omissions or additions by asterisks and brackets respectively. As Case XVIII was the one which first presented itself, the notes may appear somewhat unnecessarily full and circumstantial. In

<sup>1</sup> I am inclined to attribute the pulse-rate noted by Dr. Bridge to emotional disturbance, because I have made repeated examinations in the six cases under my care and found it only subject to normal variations.

extenuation I beg to state that when the observations were made I felt I was, perhaps, exploring a new territory and naturally wished to establish the clinical landmarks as firmly as possible. To this end, with the kind co-operation of the family physician, Dr. R. L. James, I presented (happily) the two identical cases now under discussion in person before the Neurological Section of the American Medical Association at the 1892 meeting, in Detroit, where they were seen by some of the best-known representative neurologists of this country and by Dr. James Taylor, of London.

CLINICAL NOTES OF CASE XVIII, MADE IN MAY, 1891

A business man, thirty, single, of temperate habits, good family history, except that his mother became ataxic at about thirty-three, the disease progressing steadily until she died of tubercular diarrhoea at forty-eight. The patient was active and vigorous in every way until attacked by his present disease, being rather among the foremost in all athletic sports and school work. His attention was first attracted to this disease while he was working with a surveying party in Texas, and then it was noticed by others before he himself noticed it. At that time he was twenty years old, and the ataxy was manifested by a staggering gait, which was so marked that his chief thought him intoxicated; but now he recollects that at least two years before this, or when he was eighteen years old, the draughtsmen in the office where he worked complained that he rendered their desks or drawing boards so unsteady when he leaned against them that they could not well go on with their work; and he further distinctly remembers that on a certain occasion at about this time, when in company with other young men in the country, he was quite unable to read the large letters of an advertisement at a considerable distance, though each of his companions could read them with comparative ease. He feels confident that this comparative visual defect had not always existed.

I should say here that this patient has a good English education, and that his intellectual capacity is above the average, so that, notwithstanding his affliction, he would at the present time be justly regarded as a well-informed man. He feels quite certain, too—and in this he is corroborated by his older relatives who have had an opportunity of observing him closely—that at the age of fourteen, when his voice underwent the change incident to puberty, there appeared a gradually increasing defect in utterance; his speech was slower and his syllables less definite and distinct than formerly. The ataxy has always been much more pronounced if he was fatigued, and he now remembers that at the age of eighteen he could not walk in a straight line when very tired.

There has been some progressive loss of power in the legs from the first, but this has been insignificant throughout in comparison with the ataxy. As already stated, the ataxy was first noticed in the legs and has progressed more rapidly in them than in other situations. But it has been distinct in the arms from a very early period.

\* \* \* \* \*

At no time has there been any pain or any other disturbance of sensation; neither has there been any muscular wasting, cramp, or trophic manifestations. The sphincters have not been affected, and the patient thinks sexual power has not been more impaired than can be attributed to the general decline in bodily weight and strength, which has gradually supervened in the past six years, the weight having fallen from 136 to 112 pounds in that period.

I should have stated that a tendency to choke [strangle] while eating has all along been a troublesome symptom.

\* \* \* \* \*

For several years past vision has progressively failed, so that he could best read in a dim light.

## THE PRESENT CONDITION

Patient is considerably emaciated, though he eats fairly and sleeps well. He is of medium size and well formed. The sensibility is normal; the knee-jerk is greatly exaggerated and equal on both sides; there is a slight ankle-clonus; the skin reflexes appear in the main normal, but the cremasteric and abdominal are not strongly pronounced. There is marked ataxia in nearly all voluntary movements; so that the patient can walk only when supported by an attendant, and can stand only when leaning against some solid support. Closure of the eyes does not materially increase his difficulty. The gait is such as would usually be described as cerebellar; the patient leans rather backward against his attendant and sways from side to side and all the time has an uncomfortable sense of insecurity, as if his head must fall backward to the ground. He experiences a distinct loss of power in the legs, which he thinks is even greater than could be accounted for by his general decline in bodily vigor, but the muscles are firm and well developed. All the voluntary muscular movements are slowly performed, and of this the patient is quite conscious. He cannot reach out his hand suddenly to seize any given object. The hand moves slowly and deviates several inches in various directions from the direct line that would normally be taken in such an effort. This tardiness of movement is readily noticed when the patient raises his eyes; on being addressed, or in winking, the eyelids will rise so slowly as to be suggestive of temporary ptosis, but in the end they are raised too high, so that the sclerotic is often so much exposed as to display an expression usually associated with some intense emotion, when in fact the patient is suffering from no emotional disturbance whatever. Though ataxy in the muscles that move the tongue would be difficult of conclusive demonstration, because the normal movements could hardly be definitely described (and the same might be said of the muscles that move the lips), yet a careful observer would see at a glance that the movement of both tongue and lips (especially the former in this case, because a full beard is worn) were far wider in range than usual. There is no difficulty in swallowing, excepting the tendency to choke already noticed, which might be explained by ataxy and tardiness of the muscles concerned, because no extraordinary effort has to be made, and there is no tendency for liquids to pass out through the nose.

There is a marked inco-ordination of the various muscles of facial expression, which is easily observed when the changes are going on incident to the discussion of an absorbing topic, the action being more marked now in this group of muscles and now in that, so that people casually meeting the patient frequently get the impression that there is some mental defect.

There is no spontaneous movement during sleep or waking repose, but a comparatively slight voluntary movement gives rise to very extensive and peculiar movements in muscles far removed from those required for the execution of the act. For instance, when the patient puts out his hand to take a book from a table beside which he may be sitting, the whole upper part of the body goes through a series of irregular movements highly suggestive of chorea. This is particularly the case with the head, which is somewhat inclined forward, moved from side to side, and the chin is protruded; and there is often an associated movement of the other hand. In none of these movements is there ever anything approaching a jerk.

OCULIST'S REPORT.<sup>2</sup>

Patient states that for ten years past he has suffered from some gradual impairment of vision and has had double vision at times, of not more than a few days' duration, but not for

<sup>2</sup> This case and five others of the series were thoroughly examined by Dr. W. F. Montgomery, Professor of Ophthalmology in the Woman's Medical College, whose reports I herewith append. And two of the less advanced cases (XIX

and XX) were minutely examined by Dr. Casey Wood, Professor of Ophthalmology in the Post-Graduate Medical School in this city.



## 8 DESCRIPTION OF BRAIN AND SPINAL CORD IN HEREDITARY ATAXIA

several years past. Examination shows marked ptosis when the patient is at rest, but by an effort he can raise the lids, showing sclerotic above the cornea when the eyes are directed in a horizontal plane. There is perfect co-ordination of the ocular muscles except to extreme right, where there is slight lagging of the external [lateral] rectus—not enough, however, for the production of diplopia; the lids and conjunctivæ are normal.

The pupils respond to light but very slowly; and the same is true of accommodation. Dilatation also occurs slowly on stimulation of the skin of the neck.

Vision is 20/200 in either eye. Snellen No. 5 *can* be read, though with difficulty, at eight inches in an ordinary light; much more easily in a dim light.

The ophthalmoscope shows a decided blanching of the optic discs and lessening of the caliber of the arteries, with slight but distinct atrophic changes in the retina.

There are only slight peripheral limitations of the field, and almost complete color-blindness, red only being distinguished with any degree of certainty.

[Articulation was very much affected, and in view of this fact I am greatly surprised that I did not call attention to it more explicitly in my original publication. By exercising much care and deliberation the patient was easily understood, but even then the labials were often indistinct; with less attention the articulation became somewhat confluent, with considerable elision in polysyllables. Forced utterance was conspicuous, owing, obviously, to inco-ordination between the muscles concerned in respiration, articulation, and phonation respectively.]

All the above-described symptoms advanced now more rapidly, now more slowly, up to the time of death, which occurred from simple exhaustion, emaciation having become extreme, and no intercurrent disease having been discovered either by ante- or post-mortem examination. For several days prior to death, the patient was unable to utter a sound or move a limb; the only evidence he gave that he heard what was said to him having been afforded by a slight movement of the brows.

For the last three years he had been unable to see well enough to read, but up to the end he could distinguish outlines of large figures under favoring conditions of light. The articulation had gradually failed, so that for a number of months it had been little more than a succession of grunts, intelligible only to his nurses, and vocal efforts almost invariably provoked paroxysms of coughing with distressing signs of strangulation. For fully a year before the end, and while he still had sufficient strength, he was unable to use table utensils in eating, and though he could support his weight on his legs, he could make no progress in walking on account of the extreme ataxia, even when assisted, and for more than a year had ceased making the attempt.

### CLINICAL NOTES OF CASE XX,<sup>2</sup> BROTHER OF CASE XVIII. MADE IN JUNE, 1891

Business man, twenty-four, single, well developed, cheerful disposition, and correct habits. General health always good. Did well in his studies at school and excelled in athletic sports. The ataxia appeared without exciting cause at the age of eighteen. The unsteadiness of gait and some uncertainty in use of the hands and difficulty of articulation came on together, and not until two years later was any visual defect realized. He has carried a cane for the last few months, but can walk fairly well without one, though deviating considerably from a straight line. He thinks his strength is in no way impaired, for only a few weeks ago he rode a successful tandem race with a brother who is not affected.

<sup>2</sup>Inasmuch as death occurred in this case in a comparatively early stage of the disease, both from my notes and from memory I have expanded and elaborated somewhat the record as first published. At that time it only appeared

necessary to identify the case so as to warrant its incorporation into the series. Now it is a question of correlating the anatomical findings with the symptoms.

## EXAMINATION

The knee-jerks are markedly exaggerated and all other reflexes active. There is well-marked ataxia in all four extremities. All usual movements, however, can be performed, though with evidently increased deliberation and attention. When in repose the facial muscles are considerably relaxed, giving the patient a heavy and stolid expression, which is often quite at variance with the prevailing state of mind. But during conversation the expression is similar to that already noted in the preceding case.

## OCULIST'S REPORT

There is marked static ptosis, greater in the left eye, with inco-ordinate overaction of the levator. All movements of the ocular muscles and all pupillary reflexes are sluggish. All are present, however, and in no event can nystagmus be produced. Vision is 20/80 in each eye, and Snellen No. 3 is read well enough at ten inches. Patient reads much better in a dim light. The average expert would pronounce the fundus normal, without knowing anything of the condition of vision. The color-sense is not much affected, and there is some peripheral limitation of the field of vision. All the negations mentioned in connection with the preceding case might be repeated in reference to this one.

The disease had progressed steadily and evenly, but not very rapidly, up to within fourteen months prior to death, which occurred in 1897, when symptoms of pulmonary tuberculosis made their appearance and advanced to a fatal termination. Apart from the weakness incident to the intercurrent disease, the patient was able to walk without assistance, feed and dress himself, read ordinary print, and easily make himself understood in conversation, up to the end. The knee-jerks were much more markedly exaggerated and ataxy of all voluntary movements were more pronounced than when the case was first reported. There was at no time any mental defect, diplopia, disorder of general sensation of the special senses, except vision, or the sphincters, nor was there any muscular atrophy or trophic changes.

If intercurrent disease had not intervened, it appears to me reasonable to assume that in a few years this case would have advanced to a condition of utter helplessness.

Incidentally Case XIX died from simple exhaustion within the present year, all the symptoms originally enumerated having greatly advanced and no new ones having been added.

A son of Case XVI has well-marked symptoms of the disease, which first appeared at the age of eighteen, five years before I examined him last year.

Many thanks are due Dr. R. L. James, of Blue Island, Ill.; the family of both patients, for reporting to me their condition from time to time, and for assisting at the autopsy in Case XVIII; and to Dr. C. L. Minor, of Asheville, N. C., for holding the autopsy in Case XX.

## THE ANATOMICAL FINDINGS

The brain and cord of Case XVIII were hardened in Müller's fluid and sent in this from Chicago to Baltimore, where this study was begun. The tissues from Case XX were removed by Dr. Charles L. Minor; the mass of the brain and cord was hardened in formalin, but special pieces of tissue were prepared in a series of fixing reagents, at my suggestion. I wish here to express my sincere thanks to Dr. Minor for the care and fidelity with which he followed out all the details of these suggestions.

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The tissues of these cases represent material so rarely available that it has seemed to me desirable to put on record a rather full account of the general morphology of the specimens, so that other investigators, dealing with similar cases and wishing to compare them with these, may find in this article a sufficiently detailed description. For this reason a description, which might otherwise have seemed unnecessarily prolix, will be justified.

### DESCRIPTION OF MORE ADVANCED CASE (CASE XVIII OF SANGER BROWN'S SERIES)

#### CEREBRUM OF CASE XVIII

##### HEMISPHERIUM DEXTRUM

(Figs. 3, 6, 7, 11)

**FISSURA SYLVII.**—Distance from ramus anterior horizontalis to ramus posterior ascendens, 5.5 cm. The ramus anterior horizontalis is 2 cm. long, reaching to within 0.8 cm. of the sulcus frontalis inferior. The ramus anterior ascendens is rudimentary in reality, though at first sight it would appear to be well marked. On close examination it is seen that what appears to be it is a sulcus which anastomoses above with the sulcus frontalis inferior, and superficially below with the fissure of Sylvius, though it does not cut through the opercular margin. The real ramus ascendens is situated just behind this and is scarcely visible from the surface. It makes a small incisure only between the pars opercularis and the pars triangularis of the gyrus frontalis inferior.

The fissure of Sylvius bifurcates behind into a ramus posterior ascendens 2.8 cm. long and a ramus posterior descendens 1.2 cm. long. A sulcus subcentralis posterior makes a superficial anastomosis with the fissura Sylvii behind, as does a sulcus subcentralis anterior in front of the central gyrus.

**SULCUS CENTRALIS [ROLANDI].**—The medial extremity stops 0.8 cm. short of the medial margin of the hemisphere, 1 cm. in front of the incisura sulci cinguli. The lateral extremity terminates in an obliquely placed basal piece 1.5 cm. long which runs parallel to the sulcus subcentralis anterior. In its upper part the sulcus is superficially connected with the pars medialis of the sulcus postcentralis. Otherwise it forms no anastomoses.

Relative length	- - - - -	7 cm.
Absolute length	- - - - -	8.4 cm.
Inclination of furrow with fissura longitudinalis cerebri		72°

Neither genu is well marked.

**SULCUS PRÆCENTRALIS.**—This is distinctly divided into a pars inferior, a pars superior, and a pars medialis.

*Pars inferior.*—This forms almost a semicircle with concavity backward. The lower extremity cuts deeply into the pars opercularis of the gyrus frontalis inferior, about midway between the sulcus diagonalis and the sulcus subcentralis anterior, ending 1 cm. from the fissura Sylvii. The upper extremity curves backward into the gyrus centralis anterior, just below the gyrus which connects the gyrus centralis anterior with the gyrus frontalis medius. A deep ramus anterior passes forward and upward into the gyrus frontalis medius. The pars inferior communicates superficially about its middle with the sulcus frontalis inferior, though a deep annectant gyrus separates the two sulci in the depth.

*Pars superior.*—Its lateral extremity begins in a short sagittal piece, placed just above the gyrus connecting the gyrus centralis anterior with the gyrus frontalis medius. The sulcus

extends transversally medialward for 3.2 cm., when it is interrupted by an annectant gyrus extending between the gyrus frontalis superior and the gyrus centralis anterior. At the junction of its upper and middle thirds this pars superior anastomoses with the sulcus frontalis superior in front.

*Pars medialis.*—A transverse sulcus, 2 cm. long, situated a little anterior to the upper part of the pars superior and parallel to it. It gives off a ramus sagittalis posterior, 2 cm. long, which cuts deep into the gyrus centralis anterior, reaching to within 0.7 cm. of the sulcus centralis. No part of the sulcus præcentralis reaches the medial margin of the hemisphere.

*SULCUS FRONTALIS INFERIOR.*—It begins behind in the pars inferior of the sulcus præcentralis, to extend forward for a distance of 2.4 cm., to be interrupted there by a superficial annectant gyrus extending from the pars triangularis of the gyrus frontalis inferior to the gyrus frontalis medius. It anastomoses below with the sulcus in the posterior part of the pars triangularis (false anterior ascending ramus of the Sylvian fissure). At its termination behind the annectant gyrus mentioned, it turns upward to anastomose with one of the segments of the sulcus frontalis medius. In front of the annectant gyrus is a transversal sulcus 2 cm. long, the lower part of which bisects the pars triangularis. This is probably to be regarded as the sulcus radiatus of Eberstaller. Running sagittally forward from its middle and curving around the gyrus between it and the upper extremity of the ramus horizontalis of the Sylvian fissure is a sulcus which ends in front in another obliquely placed transversal sulcus, which is to be regarded as the lateral segment of the sulcus frontomarginalis of Wernicke.

*SULCUS FRONTALIS SUPERIOR.*—It begins behind in a pars superior, 2.8 cm. from the medial margin of the hemisphere. It passes forward for 3.3 cm., where it is crossed almost at right angles by a transversal sulcus connected with the complex of the sulcus frontalis medius. The sulcus here makes a slight jog lateralward and is continued for 2 cm. farther forward, when it turns sharply medialward to end 1.4 cm. from the medial margin of the hemisphere. In front of it, between it and the medial limb of the sulcus frontomarginalis, are two more or less transversally placed compensatory sulci. At the point where it turns medialward, the sulcus frontalis superior anastomoses with a transversal sulcus which is one segment of the sulcus frontalis medius.

*SULCUS FRONTALIS MEDIUS.*—Instead of the typical sagittal sulcus, so frequently met with, this hemisphere shows two transversally placed sulci, a sulcus transversus frontalis posterior and a sulcus transversus frontalis anterior such as Teuchini and Mingazzini describe. The former anastomoses above with the sulcus frontalis superior and ends below close to the sulcus frontalis inferior; the latter anastomoses above with the sulcus frontalis superior, sends a short anterior sagittal ramus into one of the compensatory sulci in front of the sulcus frontalis superior, and lateralward bifurcates. The superior limb of bifurcation runs backward behind the gyrus frontalis medio-inferior to anastomose with the sulcus frontalis inferior, the anterior limb terminating in the gyrus frontalis medius.

*SULCUS CINGULI.*—It is of the "continuous" type, there being no subdivision into pars anterior, pars intermedius, and pars posterior. It begins in front below the genu corporis callosi, the gyrus fornicatus intervening, but it does not reach as far as the "carrefour" of Broca. It runs more or less parallel to the corpus callosum, and leaves everywhere between it and that structure a broad gyrus cinguli. Behind the lobulus paracentralis it curves upward to the margin of the hemisphere and cuts into the facies convexa for a distance of 1.2 cm. It gives off two deep radial rami into the medial surface of the gyrus frontalis superior; one, a little behind the level of the sulcus fronto-marginalis, reaches the medial margin of the hemisphere and extends for a few mm. upon the facies convexa; the other, 1.5 cm. in front of the level of the pars superior of the sulcus præcentralis, just reaches the medial margin of the hemisphere.

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**SULCUS OLFACTORIUS.**—It is situated entirely upon the orbital surface and presents no unusual features.

**SULCI ORBITALES.**—The ramus lateralis and ramus intermedius are two long, deep furrows running nearly parallel to each other throughout almost the entire extent of the orbital surface. The sulcus orbitalis transversus is interrupted by them behind. In front of their anterior extremities is a curved transversal compensatory sulcus situated just at the margin of the orbital surface. The ramus medialis is but feebly developed. The medial part of the sulcus orbitalis transversus is deep.

### ACCESSORY SULCI OF LOBUS FRONTALIS:

1. *Sulcus diagonalis operculi.*—1.8 cm. long; situated in the pars opercularis of the gyrus frontalis inferior. It forms no anastomoses.

2. *Sulcus fronto-marginalis of Wernicke.*—It is entirely separated from segments of the sulcus frontalis medius, and is represented by a Y-shaped sulcus complex, the stem of the Y being directed backward into the gyrus frontalis medius. The medial limb of the Y runs almost transversally toward the medial margin of the hemisphere, and near it undergoes tripartite subdivision. The lateral limb of the Y passes sagittally forward and also divides into three parts, the middle branch cutting into the orbital surface. The most lateral segment of the sulcus has been described along with the sulcus frontalis inferior of the sulcus radiatus.

3. *Sulcus frontalis medialis.*—This sulcus has two well-marked sagittal segments. The more posterior, 1.7 cm. long, ends anteriorly in a transversal piece 1.5 cm. long. The more anterior segment is 2 cm. in length, and gives off a short medial and a short lateral ramus. In front of it, between it and the sulcus transversus frontalis anterior of the sulcus frontalis medius, is a short compensatory transversal sulcus. The ramus posterior of the pars medialis of the sulcus præcentralis is almost in a line with the sagittal segment of the sulcus frontalis medius.

4. *Sulcus rostralis.*—A deep, well-marked sulcus running for some distance parallel to the anterior part of the sulcus cinguli. It then turns forward and runs to the medial margin of the hemisphere, to anastomose there with one of the branches of the tripartite subdivisions of the medial segment of the sulcus frontomarginalis. There is no sulcus rostralis inferior. Between the two radiating rami of the sulcus cinguli is a long, deep, sagittal sulcus on the medial surface of the gyrus frontalis superior. It gives off a number of short rami into the substance of the gyrus.

**SULCUS PARACENTRALIS.**—About the middle of the lobus paracentralis a vertical ramus from the sulcus cinguli cuts into the substance for a distance of 1.5 cm. In front of this there is a J-shaped depression and behind it a V-shaped depression in the surface.

### SULCUS INTERPARIETALIS:

1. *Sulcus postcentralis.*—This sulcus is divided into three segments: (a) a pars inferior, (b) a pars intermedius, (c) a pars superior.

The *pars inferior* is an oblique segment 2.5 cm. long, cutting into the gyrus supramarginalis above, and slightly into the gyrus centralis posterior below. Near its lower extremity it anastomoses with the well-marked sulcus subcentralis posterior of Marchand.

The *pars intermedius* 3.3 cm. long, runs almost parallel to the sulcus centralis. It is limited below by a gyrus connecting the gyrus supramarginalis with the gyrus centralis posterior. Above, it cuts into the gyrus centralis posterior, and a little above its middle it anastomoses with, or gives rise to, the sulcus interparietalis proprius.

The *pars superior* is curved somewhat like the letter S. Its medial extremity is situated behind the incisura sulci cinguli and reaches to within 0.7 cm. of the margin of the hemisphere. The sulcus is markedly convex forward about its middle. Just below this convexity, a short

sagittal sulcus, cutting across the gyrus centralis posterior, connects the pars superior with the upper part of the sulcus centralis.

2. *Sulcus interparietalis proprius*.—It begins a little above the middle of the pars intermedius of the sulcus postcentralis, and extends in a curve backward and medialward to terminate 0.5 cm. in front of the anterior limb of the U formed by the sulcus paroccipitalis. At the junction of its middle and posterior thirds it gives off a ramus medialis which extends to the margin of the hemisphere, and makes an anastomosis with one of the sulci in the præcuneus. This ramus medialis gives off a short sagittal branch which passes forward and accounts for the marked anterior convexity of the middle of the pars superior of the sulcus postcentralis. Below the sulcus interparietalis proprius anastomoses with the sulcus intermedius primus of Jensen and also gives off (a) a ramus which runs into the gyrus supramarginalis to end blindly in that gyrus, and (b) a ramus which extends into the gyrus angularis, passes through it running almost parallel to the sulcus intermedius primus, to anastomose with the sulcus temporalis superior.

3. *Sulcus paroccipitalis of Wilder*.—It is entirely separated from the rest of the sulcus interparietalis and forms the usual U around the portion of the fissura parietooccipitalis which cuts into the facies convexa. The medial extremity of the anterior limb of the U reaches to within 0.5 m. of the margin of the hemisphere. From the latter extremity of this limb a ramus sagittalis anterior is given off, which runs forward for 1.3 cm., "overlapping the posterior extremity of the sulcus interparietalis proprius." The posterior limb of the U bifurcates as it approaches the margin of the hemisphere. It forms no anastomosis with the sulcus occipitalis transversus.

**SULCUS PARIETALIS SUPERIOR.**—This sulcus runs nearly transversally on the facies convexa, being situated between the anterior limb of the U of the sulcus paroccipitalis behind and the ramus medialis of the sulcus interparietalis proprius in front. Its lateral extremity is curved a little forward. Medialward it runs to the margin of the hemisphere and anastomoses with sulci on the surface of the præcuneus.

**SULCUS SUBPARIETALIS.**—This sulcus is represented by two segments. The anterior segment anastomoses in front with the sulcus cinguli and behind turns up and anastomoses with a vertical sulcus præcunei. The posterior segment is shallow and irregular. It is limited behind by a gyrus which connects the posterior part of the præcuneus with the gyrus cinguli.

**SULCI PRÆCUNEI.**—The præcuneus presents three sulci—one anterior vertical sulcus and two antero-posterior sulci. The anterior vertical sulcus anastomoses below with the anterior segment of the sulcus subparietalis; it runs backward and upward to the margin of the hemisphere to form a superficial anastomosis with the ramus medialis of the sulcus interparietalis proprius. The two antero-posterior sulci divide the part of the præcuneus behind the vertical sulcus into three parallel gyri. The uppermost antero-posterior sulcus anastomoses at its anterior extremity with the sulcus parietalis superior which is continued upon the medial surface of the hemisphere.

**FISSURA PARIETOOCIPITALIS.**—Distance from medial margin of hemisphere to anastomosis with fissura calcarina, 3 cm.; extent upon facies convexa, 2.2 cm. On the medial surface near its upper part it anastomoses with the upper anterior-posterior surface of the præcuneus. This corresponds to an anastomosis with a sulcus on the superficies posterior lobi parietalis of the brother's brain. This brain has been hardened in Müller's fluid, and the fissura cannot be opened so as to permit of the examination of the posterior surface of the parietal lobe.

**FISSURA CALCARINA:**

Length of truncus	- - - - -	3.5 cm.
Length of fissura propria	- - - - -	3.3 cm.

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It anastomoses behind with a sulcus which passes downward, cutting off a portion which otherwise would be a part of the gyrus lingualis between it and the polus occipitalis.

**SULCUS OCCIPITALIS TRANSVERSUS.**—Above, it begins 4 mm. behind the posterior limb of the sulcus paroccipitalis, but is completely separated from it. It passes transversally downward and a little backward to anastomose with a sulcus running at right angles to it.

**SULCI OCCIPITALES SUPERIORES.**—The surface is marked by slight depressions: there are no definite sulci.

**SULCI OCCIPITALES LATERALES.**—There is one lateral sulcus, running from a point just above the polus occipitalis forward and downward to the inferior surface of the hemisphere, there to form the postero-lateral limb of the H-shaped sulcus complex. Behind the lower part of the sulcus occipitalis transversus a short sulcus runs backward and downward (almost at right angles to the course of the sulcus occipitalis transversus).

**SULCUS TEMPORALIS SUPERIOR.**—It begins in front and a little below the polus temporalis and runs backward. The gyrus temporalis superior is very narrow, averaging less than 1 cm. in thickness. The sulcus curves downward in its posterior part, owing to the ramus posterior descendens of the Sylvian fissure. Behind this region it again runs backward for a distance of 2 cm. and then turns sharply upward, curves a little forward and ends by anastomosing above with the sulcus interparietalis. In this part of its course, the sulcus runs almost parallel to the sulcus intermedius primus of Jensen, and between the two is a transversally placed gyrus, cut off from the anterior portion of the gyrus angularis.

**SULCUS TEMPORALIS MEDIUS.**—The brain has been a long time in Müller's fluid and has become brittle. Portions of the surface in the region of the sulcus temporalis medius have been broken off, and the details with regard to this sulcus are obscured. It appears to run sagittally backward for a distance of about 6 cm. and then to be interrupted. The sulci in the lobulus parietalis inferior are well preserved, as are those in the posterior portion of the temporal lobe. The arrangement is peculiar. Behind the terminal part of the sulcus temporalis superior and below the sulcus interparietalis and in front of the sulcus occipitalis transversus is a large, more or less triangular area of brain surface. Within it is a Y-shaped sulcus complex, the stem of the Y (below) corresponding in all probability to a segment of the sulcus temporalis medius (Eberstaller), *i. e.*, to Wernicke's sulcus occipitalis anterior. The anterior limb of the Y corresponds to the continuation of the stem and represents the sulcus about the extremity of which the gyrus angularis winds. The posterior limb of the Y is the sulcus about which the lobulus parietalis posterior inferior is situated. Cutting into the middle of the area from above is a short descending ramus from the sulcus paroccipitalis. This appears to be the representative of Eberstaller's sulcus intermedius secundus, as it separates the gyrus angularis in front from the lobulus parietalis posterior inferior behind.

**SULCUS TEMPORALIS INFERIOR.**—It begins in front 2.4 cm. behind the polus temporalis, is a shallow sulcus, and is interrupted by a gyrus which connects the gyrus temporalis inferior with the gyrus fusiformis. Behind, a segment runs on the inferior surface for a distance of 4 cm.; opposite, about the middle of this segment, is the incisura formerly called the incisura præ-occipitalis. It leads into an H-shaped sulcus complex in the lower part of the temporal lobe. The posterior superior limb of this complex passes upward and anastomoses with a sagittal sulcus which runs forward from the lower end of the sulcus occipitalis transversus to cut deep into the gyrus temporalis medius.

**FISSURA COLLATERALIS.**—It begins close behind the fissura rhinica, which is here only a shallow incisure and does not communicate with it. Its greatest convexity medialward corresponds to the insertion of the tip of the gyrus lingualis into the gyrus hippocampi. Around its posterior extremity an annectant gyrus passes from the gyrus lingualis to the gyrus fusiformis.

The gyrus lingualis is irregularly grooved by three sulci. The largest begins near the angle of junction of the fissura calcarina with its truncus and runs downward and backward, and then lateralward, to form the posterior inferior limb of the H-shaped sulcus complex referred to at the posterior part of the temporal lobe. Passing out of this sulcus, and at right angles to it, are two shallow sulci which anastomose in front with the posterior part of the fissura collateralis.

## HEMISPHERIUM SINISTRUM

(Figs. 4, 5, 8, 9, 10)

**FISSURA CEREBRI LATERALIS [SYLVII].**—The ramus anterior ascendens and the ramus anterior horizontalis come off from the main fissure by a common stem 1.4 mm. long. This stem bifurcates, and the ramus anterior horizontalis proper is 1.3 mm. long. The ramus anterior ascendens is 1.6 mm. long. The pars triangularis of the gyrus frontalis inferior is thus diminutive. This appears to be in part due to the very marked development of the sulcus diagonalis which cuts very deep into the pars opercularis and the lower part of the latter is driven deep into the fossa Sylvii.

**SULCUS CENTRALIS.**—The lateral extremity anastomoses with a very well marked sulcus subcentralis anterior. The latter is 2 cm. long and makes a superficial anastomosis below with the fissure of Sylvius.

Cutting into the operculum from in front in a direction at right angles to the sulcus subcentralis anterior is a little sulcus which does not appear to have been hitherto described. It is situated just below the insertion of the root of the gyrus frontalis inferior into the gyrus centralis anterior.

The sulcus centralis presents about its middle a knee projecting forward, due to an incisure into the gyrus centralis posterior from the sulcus postcentralis.

A second small knee, due to a similar cause, is seen 1.5 cm. from the medial margin of the hemisphere. The medial extremity of the sulcus reaches just to the medial margin of the hemisphere and does not pass over upon the facies medialis.

Inclination of sulcus centralis to fissura longitudinalis cerebri	70°
Relative length	7.7 cm.
Absolute length	9.7 cm.

Other than that with the sulcus subcentralis anterior, the sulcus centralis forms no anastomoses.

**SULCUS PRÆCENTRALIS.**—This presents for examination three portions: a pars inferior, a pars superior, and a pars medialis.

1. *Pars inferior.*—This forms an X-shaped complex, the anterior superior limb of the X being the ramus anterior of Eberstaller, the posterior inferior limb a ramus posterior passing beneath the insertion of the root of the gyrus frontalis medius into the gyrus centralis anterior. The other two limbs of the X represent the main direction of the sulcus. The latter is 4 cm. long, the ramus anterior 2 cm. long, and the ramus posterior 1.3 cm. long. The pars inferior anastomoses near its lower part with the sulcus frontalis inferior.

2. *Pars superior.*—It is 2.8 cm. long and "overlaps" the upper part of the pars inferior in the greater part of its extent, being situated behind it and separated from it by a superficial gyrus, a part of the gyrus frontalis medius. Near its upper or medial extremity it gives origin to the sulcus frontalis superior which passes forward at right angles to it. Its medial extremity is separated from the pars medialis by an annectant gyrus which connects the gyrus frontalis superior with the gyrus centralis anterior.

3. *Pars medialis.*—It consists of an obliquely placed H-shaped sulcus complex, situated between the medial extremity of the pars superior and the medial margin of the hemisphere.



The cross-bar of the H is the continuation of the general direction of the sulcus præcentralis. The medial extremity of the posterior side-bar of the H just reaches the medial margin of the hemisphere.

**SULCUS FRONTALIS INFERIOR.**—This begins behind in the pars inferior of the sulcus præcentralis and passes forward between the gyrus frontalis medius and the gyrus frontalis inferior. About the middle of the pars opercularis it anastomoses with a very pronounced sulcus diagonalis operculi. Beyond this point the sulcus curves upward and forward to end in the gyrus frontalis medius. Running out of it near its extremity is a sulcus which winds around the gyrus projecting upward owing to the ramus anterior horizontalis of the fissura Sylvii. This sulcus passes downward and forward to terminate in a transversal sulcus—the lateral limb of the sulcus frontomarginalis ( $fm_1$ ). In the gyrus frontalis medius, opposite the ramus anterior horizontalis of the Sylvian fissure, is a short, slightly curved transversal sulcus which probably represents the sulcus radiatus of Eberstaller on this side.

**SULCUS FRONTALIS SUPERIOR.**—It begins near the upper part of the pars superior of the sulcus præcentralis, 3.2 cm. from the medial margin of the hemisphere; it passes forward and undergoes a jog, where it is crossed by a deep transversal sulcus of the gyrus frontalis superior. It is then continued forward and medialward to within 1 cm. of the medial margin, when it suddenly curves around lateralward to terminate very close to the sulcus frontalis medius, but without actually anastomosing with the latter. This deflection of the extremity is apparently due to a deep incisure into the facies convexa from the medial margin just in front of it.

**SULCUS FRONTALIS MEDIUS.**—This is divided into two segments. The more posterior is situated in the gyrus frontalis medius, in front of the ramus anterior of the pars inferior of the sulcus præcentralis. It is 3 cm. long and runs obliquely from behind and below, forward and upward. It gives off two incisures into the brain tissue in front of it.

The anterior segment is separated from the posterior segment by a gyrus connecting the pars medialis of the gyrus frontalis medius with the pars lateralis. It begins behind in a curved transversal basal piece and extends sagittally forward for a distance of 1.5 cm. when it bifurcates to form the middle and medial segments of the sulcus fronto-marginalis ( $fm_2, fm_1$ ).

**SULCUS CINGULI.**—It begins 0.8 cm. behind the carrefour of Broca. It is nowhere interrupted. Behind it ends in an unusual way, reaching the margin of the hemisphere at a point 2.3 cm. behind the sulcus centralis. It does not pass for more than 2 mm. upon the facies convexa. Intercalated between this incisure sulci cinguli and the sulcus centralis is the pars superior of the sulcus postcentralis. The sulcus cinguli in its course gives off a number of radiating sulci which pass toward the margin of the hemisphere and into the substance of the gyrus frontalis superior.

**SULCUS OLFACTORIUS.**—This is situated entirely on the orbital surface and presents no peculiarities.

**SULCI ORBITALES.**—These are very irregular. The sulcus orbitalis transversus of Weisbach is represented in its medial half by short, obliquely placed segments, entirely separated from one another. In its lateral half it is continued deep and extends almost to the Sylvian margin of the orbital operculum.

The ramus lateralis and ramus intermedius go out from the sulcus orbitalis transversus at the same point. The ramus medialis is short and is independent of the sulcus orbitalis transversus.

#### ACCESSORY SULCI OF THE LOBUS FRONTALIS:

1. *Sulcus diagonalis operculi.*—This is very deep, and its lateral extremity together with the lower part of the whole pars opercularis of the gyrus frontalis inferior tends to be sunk into the depth of the fossa Sylvii, and to be partially hidden from view by the overlapping lobus temporalis. The sulcus anastomoses above with the sulcus frontalis inferior.

2. *Sulcus radiatus*.—*Vide supra*.

3. *Sulcus frontomarginalis* (*Wernicke*).—It consists of three segments—medial, middle, and lateral—of which the last named is independent of the other two. This lateral segment ( $fm_1$ ) forms the basal terminal-piece of the sulcus which behind is continuous with the sulcus frontalis inferior. The medial ( $fm_2$ ) and middle ( $fm_3$ ) segments represent the limbs of bifurcation of the sulcus frontalis medius. The medial segment reaches to the medial margin of the hemisphere, and the middle segment extends to within 0.7 of the lateral isolated segment.

4. *Sulcus frontalis medialis*.—There are no sagittal segments whatever to the sulcus. Instead, there are five obliquely transversal segments.

5. *Sulcus rostralis*.—This is well developed. It begins behind in Broca's carrefour and follows a course nearly parallel to the margin of the hemisphere, terminating above at the medial margin, 4 mm. behind the medial limb of the sulcus frontomarginalis.

Below the frontal pole another sulcus, representing in all probability the sulcus frontalis inferior, begins, extending for 2 cm., upon the medial surface of the hemisphere, then turns upon the facies orbitalis and runs obliquely forward and lateralward, to end in the anterior part of that surface.

**SULCUS PARACENTRALIS.**—The lobulus paracentralis presents a U-shaped sulcus. The base of the U and its anterior limb are deep; the posterior limb of the U is very shallow.

**SULCUS INTERPARIETALIS:**

1. *Sulcus postcentralis*.—This consists of three segments—inferior, middle, and superior. The inferior segment is only 1.5 cm. long, and about its middle anastomoses with the sulcus retrocentralis transversus of Eberstaller, behind it.

The middle portion is 4 cm. long and anastomoses near its upper part with the sulcus interparietalis proprius. One and a half cm. above its lower end it gives off a ramus sagittalis anterior which cuts deep into the gyrus centralis posterior, and causes a knee in the sulcus centralis.

The superior or medial segment begins 2 cm. from the medial margin of the hemisphere and extends to the margin between the sulcus centralis and the incisura sulci cinguli. Its lateral extremity curves forward into the gyrus centralis posterior. About its middle it gives off a ramus posterior which acts as a compensatory sulcus for the incisura sulci cinguli on the facies convexa.

2. *Sulcus interparietalis proprius*.—It begins in the upper part of the middle portion of the sulcus postcentralis, and follows a tortuous course backward and medialward. Behind it forms a superficial anastomosis with the U described by the sulcus paroccipitalis. About midway in its course it gives off a ramus medialis which runs medialward and forward toward the medial part of the sulcus postcentralis. The sulcus intermedius primus of Jensen passes lateralward from it between the gyrus supramarginalis and the gyrus angularis.

3. *Sulcus paroccipitalis*.—This sulcus forms the usual U about the fissura parietooccipitalis of the facies convexa. The anterior limb of the U reaches the medial margin of the hemisphere; the posterior limb of the U does not. The posterior limb of the U, together with a sulcus running lateralward from its junction with the foot of the U, makes the sulcus occipitalis transversus.

**SULCUS PARIETALIS SUPERIOR.**—It anastomoses at the medial margin of the hemisphere with the sulcus præcunei.

**SULCUS SUBPARIETALIS.**—This sulcus is separated from the sulcus cinguli in front by a gyrus 1.4 cm. wide connecting the præcuneus with the gyrus cinguli. It curves backward and downward but is separated behind from the fissura calcarina by a gyrus which connects the præcuneus with the gyrus cinguli.

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**SULCI PRÆCUNEI.**—A vertical sulcus præcunei anastomoses at the medial margin with the sulcus parietalis superior. It extends downward upon the medial surface to within 0.8 cm. of the sulcus subparietalis. Turning around its lower extremity between it and the sulcus subparietalis is a gyrus arcuatus. This gyrus is bounded in front and behind by two vertical sulci præcunei which anastomose below with the sulcus subparietalis and extend to within about 1 cm. of the medial margin of the hemisphere.

**FISSURA PARIETOOCIPITALIS.**—Distance from medial margin of hemisphere to anastomosis with fissura calcarina, 3.8 cm. Near the medial margin the fissura undergoes tripartite subdivision, one branch passing forward and running along the medial margin of the præcuneus (corresponding to the sulcus on the posterior surface of the parietal lobe of the brother's brain), a second limb cutting backward for 0.5 cm. into the facies convexa (on the anterior surface of the occipital lobe), and the third limb passing transversally upon the facies convexa. There is thus left a pyramidal depression at the margin of the hemisphere at the junction of the occipital and parietal lobes where no brain substance exists.

### FISSURA CALCARINA:

Length of truncus	-	-	-	-	-	-	-	-	3.2 cm.
Length of fissura propria	-	-	-	-	-	-	-	-	3.7 cm.

At a point 1.2 cm. from the margin of the hemisphere the fissura undergoes tripartite subdivision, the upper limb passing into the cuneus, the lower into the gyrus lingualis, and the middle (the continuation of the fissura propria) passing to the medial margin to anastomose there with a sulcus occipitalis lateralis.

**SULCI OCCIPITALES SUPERIORES.**—Behind the sulcus occipitalis transversus, and medialward from it is a transversal sulcus, short but deep, which reaches medialward as far as the margin of the hemisphere. A second superior sulcus appears as a "compensatory" sulcus posterior to the lateral extremity of the sulcus occipitalis transversus.

**SULCI OCCIPITALES LATERALES.**—There are three shallow occipital sulci on the lateral surface of the occipital lobe.

**SULCUS TEMPORALIS SUPERIOR.**—It is interrupted opposite the sulcus subcentralis anterior by a gyrus temporalis medio-superior and farther back by a second similar gyrus. The gyrus temporalis superior is narrow, being 0.8 to 1 cm. in width.

**SULCUS TEMPORALIS MEDIUS.**—The brain has been injured on this side, so that no satisfactory description of this sulcus is possible.

**SULCUS TEMPORALIS INFERIOR.**—It begins in front near the polus temporalis and passes backward, superficially interrupted, to reach the junction of the inferior with the lateral surface at the so-called incisura præoccipitalis. It then turns upward and backward upon the facies convexa; a superficial sulcus connects the sulcus temporalis medius with the sulcus temporalis inferior in this region.

**FISSURA COLLATERALIS.**—It begins in front, behind the fissura rhinica, and passes backward, being markedly convex medialward. It terminates behind at the junction of the inferior with the convex surface of the hemisphere, about 2 cm. behind the point where the sulcus temporalis inferior passes to the convex surface. The gyrus lingualis presents no typical sagittal sulcus, but instead is irregularly subdivided by two sulci which run across the gyrus at right angles to its longest diameter.

### THE CEREBELLUM (CASE XVIII)

(Figs. 1, 2, 25)

This organ looks normal in size and shape. It was divided into sagittal slabs, and sections from each of these were stained by Weigert's method. A sagittal section in the median plane is

illustrated in Fig. 25. The drawing was kindly made for me by my associate, Dr. D. G. Revell. The lingula cerebelli is not well preserved and is schematically illustrated.

The lobulus centralis, though well developed, is not very long in its vertical diameter. It does not enter into the formation of the facies superior cerebelli. There are seven *Randwülste* on its anterior surface and five on its posterior surface.

The monticulus, subdivided into culmen and declive, is well developed. As in the brain of the brother, the lobus intermedius (between the lobulus centralis and the first part of the culmen) is unusually well developed. Compare Figs. 25 and 26, with Stilling's Fig. 122. From the vertical limb of the arbor vitæ there are eight offshoots, corresponding to Stilling's C<sup>1</sup> to C<sup>8</sup> (Stilling's *erste bis dritte Wand*), while from the horizontal limb of the arbor vitæ come off the projections corresponding to Stilling's C<sup>9</sup> to C<sup>14</sup> (Stilling's *vierte bis sechste Wand*). There would seem to be no marked alteration in the number and character of the *Randwülste* on the different *Wände*, though perhaps the depressions between them are rather shallower than normal. The deep slit between the culmen and the declive is continuous lateralward with the sulcus which separates the pars anterior from the pars posterior of the lobulus quadrangularis. The folium vermis comes off from a terminal offshoot of the horizontal portion of the arbor vitæ, in relation to the lowermost portion of the declive.

The tuber vermis, well developed, is arranged around one principal stem of the arbor vitæ, which bifurcates into two main subdivisions.

The pyramis, as is usual, has a single stem of arbor vitæ which bifurcates near the periphery of the vermis inferior.

The uvula is large and well developed; it seems macroscopically quite normal.

The nodulus is perhaps relatively a little small, but otherwise presents normal appearances.

The hemispheres are well developed; the individual parts show the normal characteristic macroscopic arrangement.

#### MICROSCOPICAL EXAMINATION OF CASE XVIII

All the tissue was hardened in Müller's fluid, and the microscopical examination has therefore been practically limited to the study of Weigert preparations, and carmine specimens.

#### MEDULLA SPINALIS

##### PARS LUMBALIS (Fig. 44):

##### DIMENSIONS

Antero-posterior diameter	- - - - -	0.7 cm.
Transverse diameter	- - - - -	0.84 cm.

*Radices anteriores et posteriores.*—These are small and contain fewer fibers than normal. The connective tissue of the endoneurium is increased in amount. In the anterior roots a very large proportion of the fibers are small. The number of large fibers appears to be diminished.

*Pia mater.*—This is thickened, as in the other case, measuring as much as 0.159 mm. in thickness in places.

##### *Subphial layer of neuroglia or so-called "glial sheath."*

Maximum thickness opposite anterior horn	- -	0.045 mm.
Maximum thickness opposite funiculus lateralis	-	0.2 mm.
Average thickness over funiculus posterior near medial line	- - - - -	0.061 mm.
Average thickness over funiculus posterior near entrance of posterior root	- - - - -	0.053 mm.
Maximum thickness over Lissauer's fasciculus	- -	0.133 mm.

*Sections stained with Weigert's myelin sheath stain and with iron hæmatoxylin stain.*

—The tissue does not stain satisfactorily, owing to imperfect hardening. There is no difficulty, however, in coming to a decision as to the state of the various fasciculi.

The different fasciculi of the funiculi anteriores and of the funiculi laterales present no areas of degeneration. The only alterations noticeable in these preparations are met with in the funiculi posteriores. Here there is tolerably symmetrical degeneration of both funiculi. The lightening corresponds in general to the area designated by Flechsig as the "middle root zone." The degeneration is most marked in the middle of the dorsal funiculus at a level drawn through the substantia gelatinosa of the two sides. It extends dorsalward, but leaves Flechsig's dorsal root zone unaffected, the latter sending a triangular process in between the degenerated area and the posterior median septum on each side. Ventralward the degeneration extends almost to the substantia grisea, but the white matter of the dorsal funiculi nearest the gray matter (corresponding to Flechsig's ventral root zone) is less affected. Though the number of fibers is greatly diminished in the lightened areas, the degeneration is only partial. Nowhere have all the fibers disappeared. The lightening is due to the disappearance of a portion of the medullated fibers and to a corresponding increase in the neuroglia. The distribution of the lesion is well shown in Fig. 44.

The gray matter, as a whole, looks less voluminous than normal; the substantia gelatinosa is less deep than in healthy specimens. A section at the level of T XII was kindly measured by Professor H. H. Donaldson, who found the area of the white matter 9.375 cm.<sup>2</sup> and the area of the gray matter 2.186 cm.<sup>2</sup>, the ratio being 1 : 4.3.

*Sections stained with Van Gieson's preparations and with uranium carmine.*—The excessive connective tissue in the anterior and posterior roots and in the pia mater is collagenous, white, fibrous tissue staining intensely in acid fuchsin. In the degenerated area in the funiculus posterior the neuroglia is seen to be diffusely increased and stained of a pink or rose-red color, single yellow myelin sheaths or small groups of myelin sheaths standing out sharply on account of their isolation. There is a tendency to the formation of stripes of neuroglia, radiating dorso-medialward from the dorso-medial surface of the substantia grisea of the cornua posteriores. The blood-vessels are larger in these glia bands, and many of them have unusually distinct fibrous tissue coats which stain in acid fuchsin. The fibers which remain undegenerated in the area in which the neuroglia is increased vary in size. I get the impression, however, that the finer fibers of the area have suffered more than the coarser fibers from the disease-process.

The anterior horn cells appear to be reduced somewhat in number. The methods employed do not permit of any satisfactory description of the internal morphology of these cells. The central canal is obliterated, its place being taken by an irregular aggregation of proliferated ependymal cells.

PARS THORACALIS (UPPER PART) (Fig. 43).—The cord has been injured somewhat on removal, but the effects of the injury can be easily distinguished, so that there is no doubt as to the actual changes in the tissues.

#### MEASUREMENTS

Antero-posterior diameter	- - - - -	6 mm.
Transverse diameter	- - - - -	7.5 mm.

The pia mater is thickened as in the lumbar region. The subpial neuroglia layer is in general delicate in the pars thoracalis.

*Sections stained with Weigert's myelin sheath stain or with iron hæmatoxylin.*—The anterior and posterior roots are small and the connective tissue is exaggerated in them, though not so markedly as in the roots of the lumbar nerves.

There are no degenerated areas visible in the funiculi anteriores.

The funiculi laterales present marked alterations. On each side the direct cerebellar tract of Flechsig is almost completely degenerated. Only here and there can a fiber of large caliber be made out in the area corresponding to this fasciculus. A good many fine fibers are visible in that portion of the area immediately adjacent to the main part of the pyramidal tract, but these are doubtless themselves fibers of the pyramidal tract which are normally mixed in with the fibers of the direct cerebellar tract. This marked area of degeneration of the direct cerebellar tract is situated as always in the dorso-lateral portion of the funiculus. The ventro-lateral region of the funiculus shows no distinct area of lightening, though the large fibers of that region seem to be less numerous than normal. It is therefore probable that the ventro-lateral cerebellar tract of Gowers has suffered some, though the chief lesion in the funiculi laterales is undoubtedly an extensive degeneration of the dorso-lateral direct cerebellar tract of Flechsig.

The pyramidal tract is not degenerated, nor can I make out any alteration in the fasciculus lateralis proprius.

In the funiculi posteriores there are some partial degenerations tolerably definitely localizable. The partial degeneration assumes approximately the shape of the letter W. The lateral limbs of the W correspond to regions in the funiculi cuneati of the two sides; the medial limbs, to the regions in the fasciculi graciles of the two sides. The degeneration is fairly symmetrical. That in the fasciculus cuneatus of each side occupies a rather broad strip corresponding to about one-third of the width of the fasciculus at its broadest part (opposite the substantia gelatinosa). Dorsalward the strip becomes narrower and ceases at some little distance from the dorsal periphery. Ventralward the degenerated area in the fasciculus cuneatus approaches the median line and fuses with that of the fasciculus gracilis. Though this degeneration passes far ventralward, almost to the substantia grisea, it does not quite reach the latter. Flechsig's ventral root zone remains almost entirely undegenerated. A strip of some breadth intervenes lateralward from the degenerated area between it and the substantia grisea of the dorsal horn.

In the fasciculus gracilis the degenerated strip is narrow; rather fusiform, and more medially than laterally situated. The strip is widest and the degeneration in it most intense about midway between the dorsal periphery and the ventral extremity of the fasciculus. The degenerated area does not extend dorsalward as far as the posterior surface, but growing gradually less distinct, terminates at some distance from this. Ventralward the strip fuses laterally on each side with the degenerated strip in the fasciculus cuneatus.

It will be noticed that the description of this degeneration corresponds very closely in a negative way to Trepinski's description in a positive way of his third foetal system in the thoracic portion of the cord. (Cf. Fig. 290, p. 434, of my book on *The Nervous System*.) It also reminds one forcibly of Flechsig's description of the degeneration in a case of incipient tabes in the upper portion of the thoracic cord; described by him in the *Neurologisches Centralblatt* for 1890.

Lissauer's fasciculi are intact on both sides.

These specimens reveal further important alterations in the white fibers which enter the gray matter of the cord. Thus, while Waldeyer's nucleus of the dorsal horn still stains black and is rich in fine medullated fibers, the nucleus dorsalis on each side is almost entirely devoid of them. There is a marked contrast between the nucleus dorsalis of this case and that of the case to be subsequently described, where many of the fine medullated fibers of Clarke nucleus are still retained. The substantia gelatinosa is still crossed with many radiating bands of fine black fibers, and medullated fibers pass forward toward the anterior horns of gray matter.

*Sections stained with van Gieson's method and with Schmaus's uranium carmine.*—Specimens stained by these methods demonstrate very beautifully the proliferation of the neuroglia in the areas of partial degeneration in the dorsal funiculi. In the place of the fibers which

have degenerated there are dense masses of rose-pink staining neuroglia. The increase in the neuroglia is much more marked in the fasciculus cuneatus than in the fasciculus gracilis. Here, too, as in the pars lumbalis, there is a tendency to the arrangement of the neuroglia in coarse strands radiating out from the dorsal horns of gray matter. In the areas in which the neuroglia is increased, the blood-vessels are larger and have thicker walls than normal. I get the impression that in the areas of partial degeneration the small fibers have suffered more than the fibers of larger caliber.

The appearance of the degenerated area in the lateral funiculus corresponding to the direct cerebellar tract is very different from that of the partial degeneration of the dorsal funiculi. While in the latter case one is impressed with the proliferation of the glia, and the compression of the fibers, in the former it is the great loss of fibers and the spaces formerly occupied by them which strikes one, rather than the increase in the glia. There has probably been an increase in the glia substance in the region of the direct cerebellar tract, but it is not in the form of solid masses as in the dorsal funiculi, but instead forms a coarse, much perforated network. It looks as though the fibers had simply been dissolved out, and that the glia and ependymal framework normally present had been swollen or slightly exaggerated in amount. Undoubtedly the process involving the glia here is a wholly different one from that which affects the glia in the dorsal funiculi.

The cells in the nucleus dorsalis have almost entirely disappeared. Here and there a single cell can be made out, probably corresponding to the isolated large fibers still preserved in the direct cerebellar tract.

**PARS CERVICALIS (INTUMESCENTIA) (Fig. 42):**

**DIMENSIONS**

Antero-posterior diameter	- - - - -	6.5 mm.
Transverse diameter	- - - - -	11.0 mm.

*Sections stained with Weigert's myelin sheath stain or with iron hæmatoxylin.*—I can make out no alterations in the funiculi anteriores. In the funiculi laterales the dorso-lateral areas (direct cerebellar tract) are very extensively degenerated on both sides, though a few more large fibers are preserved on one side than on the other. The ventro-lateral areas (region of Gowers's tracts) do not contain so many large fibers as normally, but there is no marked lightening of this region.

In the funiculi posteriores there are extensive areas of partial degeneration, giving rise to moderate lightening in the ventral half of the fasciculus gracilis and in the ventral and middle portions of the fasciculus cuneatus. The dorsal third of the fasciculus gracilis is entirely free from the degeneration process, and the periphery of the fasciculus cuneatus is not involved. Ventralward the area of degeneration of the fasciculus gracilis fuses with that of the fasciculus cuneatus. Close to the posterior commissure and gray matter of the dorsal horn the fibers are better preserved. The degeneration is only partial in the lightened areas and is, perhaps, most intense in the middle of Burdach's and in the ventral third of Goll's fasciculus. The anterior and posterior white commissures present no noticeable alterations.

*Sections stained with van Gieson's method and with Schmaus's uranium carmine.*—The decrease of white fibers of the nerve roots, especially in the radices anteriores, is distinctly visible. There are fewer nerve cells in the anterior horns than normal. The proliferation of glia in the degenerated areas in the funiculi posteriores is pronounced. The region of the direct cerebellar tract in the funiculi laterales stains red and the tissue is not dense; one sees there a much perforated network with only a few medullated fibers retained.

**MEDULLA OBLONGATA AND PONS VAROLI.**—The small size of the medulla oblongata and pons

is evident macroscopically. The length of the pons in the ventral middle line is 2.2 cm., transverse distance between nervi trigemini of two sides 3 cm., maximal thickness 2.5 cm. The depression between the pyramis and the oliva is more marked than usual. The lower part of the medulla has been injured on removal of the brain, so that the nucleus funiculi cuneati and the nucleus funiculi gracilis cannot be examined except in their upper parts; there, however, they are smaller than normal; the bulk of the upper part of the nucleus funiculi cuneati is certainly diminished. There is slight thinning of fibers in the stratum interolivare lemnisci and also higher up in the lemniscus medialis. The pyramids are well developed and stain normally. The nuclei arcuati are small. The layer of gray matter of the nucleus olivaris inferior looks thinner than normal and the number of cells present in it somewhat reduced. The fibræ olivo-cerebellares are less prominent than normal, as are also the fibræ arcuatæ externæ. The corpus restiforme is rather smaller than normal, and shows a thinning of fibers with network-like increase of neuroglia. The nuclei nervi hypoglossi are smaller than normal, and contain correspondingly fewer cells. The nucleus on one side contains fewer cells than that on the other. The tractus solitarii are present, and show no recognizable alterations. The tractus spinalis nervi trigemini looks normal. The lateral region of the formatio reticularis and the nuclei laterales are much less developed than in normal specimens, and the notch dorsal to the olive is deepened as in Menzel's case. The mass of substantia gelatinosa medial to the tractus spinalis nervi trigemini is small.

In the pons the diminution in size is general, but the various structures appear to be fairly evenly developed in relation to one another. The fasciculi longitudinales pontis and fibræ transversæ pontis look normal and the great nuclei pontis are relatively well represented. The masses of gray matter in the pars dorsalis pontis are not markedly altered in appearance.

#### THE CEREBELLUM

##### 1. SAGITTAL SECTION IN MEDIAN PLANE (Fig. 25):

###### a) *The arbor vitæ.*

(1) *Main vertical limb.*—This medullary stem receives as usual the bands of medullated fibers (laminæ medullares) from the first three *Wände* of the monticulus and part of those from the fourth *Wand*. Part of the fiber bands enter the main vertical limb from in front, part from behind. The main vertical limb descends to form with the horizontal limb the fiber-mass of Stilling's corpus trapezoideum.

(2) *Horizontal limb.*—This medullary stem receives as usual the vertical laminæ medullares, corresponding to Stilling's fifth and sixth *Wände* and most of the white fibers from Stilling's fourth *Wand*. It runs forward to enter the corpus trapezoideum from behind. Below it receives the *Markaste* from tuber, pyramis and uvula.

(3) *Stilling's corpus trapezoideum.*—In the median plane this is of minimum dimensions. Its configuration and contents, however, correspond to Stilling's descriptions of the normal cerebellum. It receives directly or indirectly the laminæ medullares from all the subdivisions of the vermis superior and inferior.

b) *The great anterior decussation-commissure.*—This includes the fibers passing from the left lateral half of the vermis into the right half and *vice versa* at the base of the lingula and of the lobulus centralis, and throughout the whole extent of the main vertical limb of the arbor vitæ. It is Stilling's "*grosse vordere Kreuzungs-Commissur*."

c) *The posterior decussation-commissure.*—This includes the fibers which run transversely from one side of the worm to the other at the posterior end of the horizontal limb of the arbor vitæ, immediately after entrance of the *Markaste* of the declive, folium, and tuber.

d) *The nucleus of the roof (nucleus fastigii).*—This nucleus, Stilling's *Dachkern*, is in



## 24 DESCRIPTION OF BRAIN AND SPINAL CORD IN HEREDITARY ATAXIA

its normal position in the lower half of the trapezoid mass of white matter behind the lingula and the lobulus centralis. Its dimensions are those ordinarily met.

2. SAGITTAL SECTION 1 CM. LATERAL FROM SAGITTAL MEDIAN PLANE.—The section passes through the ala lobuli centralis, the lobulus quadrangularis, the lobulus semilunaris superior, the lobulus inferior, the lobulus biventer and the flocculus. The appearance corresponds closely to B. Stilling's Vol. III (atlas), Plate VIII *b*, Figs. 65 and 66.

The transverse, vertical and obliquely cut fibers in the central white substance correspond in numbers and position to normal descriptions.

The dentate nucleus (*nucleus dentatus*) (Stilling's corpus dentatum s. ciliare cerebelli) in its more medial part is seen in the section. No trace (except one minute island of cells) of the nucleus globosus or of the nucleus emboliformis is present as the section passes just lateralward from these gray masses. In this section the ellipse formed by the cut section of the dentate nucleus is open in the middle below, and also in its anterior superior region. The typical tortuous appearance of the section of the nucleus is present; this is due to the *Zacken* and *Gegenzacken* of Stilling. The greatest length of the nucleus dentatus in the sagittal direction is 18 mm. The cell-bodies in the gray matter of the nucleus dentatus as seen in sections at this level are reduced to almost one-quarter the normal number. Within the nucleus dentatus is a mass of nerve-fibers for the most part cut obliquely. But instead of the compact, solid masses of medullated fibers usually seen, the fibers are somewhat thinned and under the loop the Weigert preparation shows lightening; the lightening is tolerably general, though it is most pronounced perhaps at the upper part and the posterior part of the intranuclear fiber-mass. In this fiber-mass are ten or twelve sections through large blood-vessels visible to the naked eye; these vessels are surrounded by large clear spaces.

The fibræ semicirculares cerebelli, or Stilling's *halbzirkelförmige Faserzüge*, lying in that part of the central white matter of the cerebellum which is situated between the upper margin of the nucleus dentatus and the bases of the various *Wände* of the lobulus quadrangularis show no alteration that can be made out in the Weigert sagittal section at this place. Between the fibræ semicirculares and the bases of the *Wände* of the lobulus quadrangularis the *guirlandenförmige Fasern* of Stilling are present and at the bases of the *Wände* themselves the bundles of fibers cut in cross-section, so characteristic of the normal cerebellum, are to be seen in what appear to be normal numbers.

In this central white matter (corpus medullare) beneath the base of the lamina medullaris of the posterior part of the lobulus quadrangularis, there is a slightly lightened area in the Weigert section; the lightening may be due to a slight thinning of the fibers in this area.

The white matter of the flocculus is well developed and stains normally, as do the laminæ medullares of the other portions of the inferior surface of the hemisphaerium cerebelli. The section passes through a part of the corpus restiforme. A histological study of the substantia corticalis in sagittal sections at this plane reveals no striking alterations. The molecular layer, layer of ganglion cells, and granular layer show normal relative development.

### DESCRIPTION OF THE LESS ADVANCED CASE (CASE XX)

#### CEREBRUM

##### HEMISPHERIUM DEXTRUM

(Figs. 12, 14, 16, 17, and 18)

##### FISSURA CEREBRIS LATERALIS [SYLVII]:

*Truncus*.—The polus temporalis does not cover more than the middle half of the pars orbitalis of the gyrus frontalis inferior; it fails to reach anteriorly as far as the sulcus orbitalis transversus by a distance of 0.5 cm.

*Fissura propria*.—The angle formed with the longest antero-posterior diameter is  $13^{\circ}$ . The linear distance from the junction with the ramus anterior horizontalis to the junction with the ramus posterior ascendens is 6.2 cm. The ramus posterior ascendens goes off at an angle of  $71^{\circ}$  and is 2.0 cm. in length. The ramus posterior descendens is 1 cm. in length.

*Ramus anterior horizontalis and ramus anterior ascendens*.—These arise by a common stem, the horizontal ramus cutting deep into the gyrus frontalis inferior and reaching to within 0.7 cm. of the sulcus frontalis inferior. It is very prominent on the lateral surface of the cerebrum. The ascending anterior ramus is only 0.5 cm. long. It passes upward and backward from the common stem. It is inconspicuous on the lateral surface, especially in comparison with the sulcus diagonalis operculi.

**SULCUS CENTRALIS [ROLANDI]**.—The medial extremity extends beyond the junction of the convex and medial surfaces of the hemisphere, encroaching for a few millimeters upon the medial surface. The genu superior is more marked than the genu inferior. The sulcus turns backward into the paracentral lobule and stands in the typical relation to the extremity of the sulcus cinguli (*cf.* Eberstaller). The lateral extremity of the sulcus is situated 1.5 cm. from the fissure of Sylvius. Between it and the fissure of Sylvius is intercalated an oblique sulcus (the course of which is almost perpendicular to that of the lower third of the sulcus centralis). It is 2.3 cm. in length and is bifurcated at its lower extremity. This is evidently a special case of Eberstaller's *ctr.-Furche* (Retzius's "sulcus subcentralis anterior").

No anastomoses with other sulci.

Relative length	- - - - -	7.8 cm.
Absolute length	- - - - -	10.0 cm.

Inclination of sulcus with fissura longitudinalis cerebri, measured on a line drawn from the point where the sulcus cuts the medial margin to the lower extremity of the sulcus,  $66^{\circ}$ .

#### LINEAR DISTANCE OF THE MEDIAL END OF THE SULCUS

From the frontal pole	- - - - -	120 mm.
From the occipital pole	- - - - -	95 mm.

that is, as 56:44.

#### LINEAR DISTANCE OF LATERAL END

From frontal pole	- - - - -	89 mm.
From occipital pole	- - - - -	112 mm.

#### SULCUS PRÆCENTRALIS:

1. *Pars inferior (sulcus præcentralis inferior of Eberstaller)*.—The pars inferior is superficially continuous with the pars superior, though the two segments are distinctly separated from one another by a gyrus in the depth. The lower extremity of the pars inferior reaches to within 0.9 cm. of the margin of the Sylvian fissure, making a deep incisure in the interval between the sulcus subcentralis anterior and the sulcus diagonalis. The pars inferior about its middle forms a deep anastomosis with the posterior extremity of the sulcus frontalis inferior. At first sight there would seem to be no ramus anterior of Eberstaller (ramus horizontalis of Cunningham) on this side, but on closer examination and comparison with the opposite hemisphere it is seen that in reality such a ramus exists. It is, however, completely separated from the main sulcus by an annectant gyrus extending between the lateral and medial roots of the gyrus frontalis medius.

2. *Pars superior (sulcus præcentralis superior of Eberstaller)*.—This pars is a transversally placed foot-piece to the sulcus frontalis superior with which it forms a deep anastomosis. Its lateral extremity extends backward into the gyrus centralis anterior, posterior from the medial extremity of the pars inferior and reaches within 0.5 cm. of the sulcus centralis. The

superficial anastomosis of the pars superior with the pars inferior is situated 1 cm. above and in front of this lateral extremity. The medial extremity of the pars superior fails (by 0.5 cm.) to reach the medial border of the hemisphere, apparently owing to the very marked development there of a sulcus which is probably to be identified with the sulcus præcentralis medialis of Eberstaller. The latter sends two rami forward, one behind, the other in front of, the medial extremity of the sulcus præcentralis superior. The more posterior and lateral ramus is separated from the medial extremity of the sulcus præcentralis superior by a narrow gyrus, only 0.4 mm. in thickness. Passing backward from about the middle of the sulcus præcentralis superior, and cutting deep into the substance of the gyrus centralis anterior for a distance of 1.5 cm. is a well-marked branch (ramus sagittalis posterior of Retzius). This is in almost a direct line with the sulcus frontalis superior, and is probably to be recognized as a part of the latter sulcus, instead of as a part of the sulcus præcentralis. The sulcus complex formed by the sulcus præcentralis superior and the sulcus frontalis superior is an unusually good example of the "cruciform type" which has frequently been described.

3. *Pars medialis (sulcus præcentralis medialis of Eberstaller).*—This commences on the medial surface in about the middle of the paracentral lobule as a ramus,<sup>4</sup> which begins 0.7 cm. above the sulcus cinguli and extends vertically upward for a distance of 1.3 cm., then turns at an angle of 108° forward and slightly medialward for a distance of 1.4 cm., where it bifurcates on the medial margin of the hemisphere to form two slightly curved terminal prongs. One of these runs forward along the edge of the hemisphere for a distance of 1.1 cm.; the other, lateralward for the same distance, to terminate in the gyrus centralis anterior close behind the medial portion of the sulcus præcentralis superior (*vide supra*).

**SULCUS FRONTALIS INFERIOR.**—This sulcus is completely interrupted by a superficial annectant gyrus 0.7 cm. broad, which connects the pars triangularis of the gyrus frontalis inferior with the gyrus frontalis medius. This gyrus evidently corresponds to Eberstaller's *vordere Tiefenwindung*. The posterior part of the sulcus anastomoses behind with the pars inferior of the gyrus præcentralis, extends, tortuous, 1.7 cm. forward and somewhat lateralward, and terminates in a transversal sulcus which cuts above into the gyrus frontalis medius to within 0.4 cm. of the sulcus frontalis medius, and below into the pars triangularis to within 0.8 cm. of the ramus anterior horizontalis of the Sylvian fissure.

The anterior part of the sulcus frontalis inferior begins as an oblique slit, 1.7 cm. long and directed from behind and lateralward (in the pars triangularis) upward and medialward into the gyrus frontalis medius. From a little above the middle of this oblique piece, the main sulcus runs forward for a distance of 1 cm. and there gives off a short medial ramus which cuts into the gyrus frontalis medius; it then turns abruptly (angle of 85°) lateralward, to end in the middle of a sagittally placed terminal sulcus, 1.5 cm. in length. The latter is situated almost exactly on the border of the orbital surface. The anterior extremity of this terminal piece lies 0.7 cm. in front of the lateral extremity of the sulcus frontalis marginalis. It seems probable that we have here to deal with a fusion of the anterior portion of the sulcus frontalis inferior with the sulcus radiatus of Eberstaller.

**SULCUS FRONTALIS SUPERIOR.**—The anastomosis of this sulcus with the pars superior of the sulcus præcentralis has already been noted. This occurs at a point 2 cm. distant from the medial margin of the hemisphere. The sulcus is continued back into the gyrus centralis anterior. I agree with Eberstaller that this prolongation (called by Retzius the ramus sagittalis posterior of the sulcus præcentralis) belongs in reality to the sulcus frontalis superior.

The main sulcus follows a tortuous course forward and medialward and terminates in front about 2.5 cm. from the margin of the hemisphere. At about the junction of its posterior

<sup>4</sup> This ramus may represent a sulcus paracentralis proprius.

and middle thirds it gives off a ramus which cuts lateralward and slightly backward deep into the gyrus frontalis medius. Between this ramus and the sulcus centralis is an isolated gyrus which, at first sight, might be thought to belong to the gyrus frontalis superior, but which must be regarded as a part of the medial portion of the gyrus frontalis medius. The sulcus frontalis superior is nowhere interrupted by annectant gyri, nor does it form anastomoses other than that with the sulcus præcentralis.

**SULCUS FRONTALIS MEDIUS.**—This is a pronounced sagittal sulcus, almost completely dividing the gyrus frontalis medius into two halves, and so establishing the "four-convolutions" type of the frontal lobe in this hemisphere. It begins behind close to the inferior portion of the sulcus præcentralis, as a shallow piece which may, in reality, represent a detached ramus anterior of the inferior præcentral sulcus. One and five-tenths cm. in front of its posterior extremity it is crossed by a transversal sulcus 1.5 cm. long; then becoming very deep, it extends 1.5 cm. farther forward and bifurcates into a medial ramus 1 cm. long and a lateral (more sagittal) ramus which extends for a distance of 2 cm. and is then interrupted in the depth by an annectant gyrus connecting the pars medialis with the pars lateralis of the gyrus frontalis medius. In front of this gyrus in the depth, the sulcus makes a deep cut backward and medialward into the pars lateralis of the gyrus frontalis medius, while farther onward it bifurcates to form the two limbs of the sulcus frontomarginalis of Wernicke.

**SULCUS CINGULI.**—This sulcus belongs to Eberstaller's "continuous type."

The pars anterior begins under the front part of the genu corporis callosi, passes forward, then upward, and later backward. It is very shallow in all parts of its course and runs unusually close to the corpus callosum, making a very narrow gyrus fornicatus in front. At its posterior extremity it curves up into the medial surface of the gyrus frontalis superior, to terminate 0.7 cm. from the margin of the hemisphere.

The main course of the pars anterior is, however, continued backward as the pars intermedius, the latter being much more widely separated from the corpus callosum than is the pars anterior. Behind, the pars intermedius becomes somewhat irregular and goes over into the pars posterior. The ascending portion of the latter undergoes a Y-shaped bifurcation (*lilienartig gegabelt*). The anterior limb of the Y passes up to the medial margin of the hemisphere and terminates a few millimeters behind the medial extremity of the sulcus centralis. The posterior limb of the Y terminates in the præcuneus without reaching the margin of the hemisphere.

**SULCUS OLFACTORIUS.**—Deep; begins 1 cm. from the medial margin, behind. Bifurcates at posterior extremity. No anastomoses. Length, 4.4 cm. Passes forward and medialward. Reaches medial margin and extends 0.7 cm. upon medial surface.

**SULCUS ORBITALIS.**—This is of the H type.

The sulcus orbitalis transversus, ramus lateralis, ramus medialis, and ramus intermedius are all present. The ramus intermedius forms a superficial anastomosis with the ramus lateralis. Running backward from the sulcus orbitalis transversus into the gyrus frontalis inferior is a sagittal ramus. Its course is nearly parallel to that of the medial extremity of the sulcus orbitalis transversus.

#### ACCESSORY SULCI OF THE LOBUS FRONTALIS:

1. *Sulcus diagonalis operculi.*—Very deep; typical direction; extends 2.4 cm. on lateral surface and 1 cm. on inferior surface of pars opercularis. No anastomoses.

2. *Sulcus radiatus.*—Cannot be distinctly made out. If present at all, it belongs to the sulcus-complex, composed of that part of the sulcus frontalis inferior which lies along the front part of the base of the pars triangularis and anastomoses farther forward with the lateral segment of the sulcus frontomarginalis of Wernicke.

3. *Sulcus frontomarginalis of Wernicke.*—The lateral portion ( $fm_1$ ) anastomoses with

the anterior extremity of the gyrus frontalis inferior; the middle portion ( $fm_1$ ) is 1.8 cm. long, is entirely in the facies convexa and is the lateral limb of bifurcation of the anterior extremity of the sulcus frontalis medius, but the posterior of the two is evidently the continuation of the sulcus frontalis medialis, the anterior being partially separated by a deep annectant gyrus.

4. *Sulcus frontalis medialis and other accessory sulci of the gyrus frontalis superior.*—The gyrus frontalis superior is divided into two distinct gyri by a well-developed sulcus frontalis medialis (medial frontal sulcus of Cunningham), a deep sagittal sulcus, 4 cm. in length. There are no accessory transversal sulci. The marked development of the sulcus frontalis medialis gives rise to that rare appearance known as the "five-convolution" type of frontal brain.

5. *Sulcus rostralis.*—Very deep and well marked. A far more conspicuous sulcus than the pars anterior of the sulcus cinguli. It begins in the lower part of the sulcus rostralis transversus and, following a course almost perpendicular to the sulcus cinguli, terminates in the gyrus frontalis superior at a point vertically above the anterior extremity of the corpus callosum. A number of radially directed furrows run out from it into the gyrus frontalis superior toward the margin of the hemisphere.

6. *Sulcus rostralis inferior.*—Well developed; 3.5 cm. long; deep and continuous. Eberstaller says of this sulcus: "Gut ausgebildet findet sie sich nur an sogenannten windungs-, bezw. furchenreichen Gehirnen."

7. *Sulcus olfactorius transversus.*—Shallow; entirely on medial surface of hemisphere. Anastomoses with the sulcus rostralis inferior.

8. *Sulcus paracentralis.*—Cf. description of sulcus praecentralis medialis.

#### SULCUS INTERPARIETALIS:

1. *Sulcus postcentralis.*—This sulcus is continuous and does not anastomose with the sulcus interparietalis proprius. Its lateral extremity anastomoses with the sulcus subcentralis posterior of Marchand. Its medial extremity turns backward and ends 1 cm. from the medial margin of the hemisphere. The sulcus is deep throughout, there being no annectant gyri in the depth.

2. *Sulcus interparietalis proprius.*—This begins in an X-shaped sulcus-complex. The lateral posterior limb of the X, which cuts across the gyrus angularis, is separated from the rest of the complex by an annectant gyrus in the depth. The medial posterior limb of the X corresponds to the main course of the sulcus separating the lobulus parietalis superior from the lobulus parietalis inferior. The medial anterior limb of the X measures 2 cm. in length, the lateral 1.5 cm. The medial posterior limb (main sulcus) passes backward and slightly medialward for 2 cm., where it bifurcates. The medial limb of bifurcation cuts into the lobulus parietalis superior, the other limb communicating superficially with the posterior part of the sulcus interparietalis (the so-called paroccipital sulcus of Wilder).

3. *Sulcus paroccipitalis* of Wilder.—This forms a U around the fissura parietooccipitalis of the facies convexa. Its anterior extremity begins 3 cm. from the margin of the hemisphere, 0.7 cm. in front of the fissura parietooccipitalis. Its posterior extremity is 1.3 cm. from the margin of the hemisphere where it appears to anastomose with the sulcus occipitalis transversus, but on examination it is found to be separated from it by an interrupting gyrus which reaches almost to the surface.

SULCUS PARIETALIS SUPERIOR.—The sulcus appears as a direct transversal continuation of the sulcus praecunei upon the facies convexa. After an extent of 1.5 cm. it ends in a sagittal cross-piece 1 cm. long. It does not anastomose with the sulcus postcentralis or with the sulcus interparietalis.

SULCUS SUBPARIETALIS AND SULCUS PRAECUNEI.—The former forms a superficial anastomosis with the pars posterior of the sulcus cinguli, passes backward for 2.5 cm., and bifurcates into a posterior and an inferior terminal ramus. A little behind its middle it anastomoses with the sulcus praecunei.

**FISSURA PARIETOOCIPITALIS.**—Distance from medial margin of hemisphere to point of junction with the truncus of the calcarine fissure, 3.8cm. The fissure extends 1.5cm. on the facies convexa and then bifurcates into two short superficial terminal rami. On opening up the fissure from the medial surface, the superficies posterior lobi parietalis comes into view. A typical gyrus cunei of Ecker exists. Near the medial border of the surface a deep tortuous sulcus passes upward and about 1cm. from the medial margin of the hemisphere reaches the facies medialis; it then turns sagittally forward through the præcuneus for a distance of 2cm. and then turns vertically upward 1cm. behind the sulcus præcunei to terminate near the medial margin of the hemisphere.

The portion of the superficies posterior lateral from the sulcus just described is marked near the bottom of the fissura parietooccipitalis by a transverse sulcus above which is a V-shaped sulcus. The lateral limb of this V corresponds to the anterior limb of bifurcation of the fissura parietooccipitalis as seen from the facies convexa. There thus arises a diminutive lobulus parietooccipitalis in the sense of Retzius at the lower end of the fissure. It comes, however, in the domain of the gyrus arcuatus posterior of the lobulus parietalis.

**FISSURA CALCARINA.**—Length of truncus, 1.8cm. The fissure extends 3.8cm. beyond the truncus, terminating behind 0.5cm. from the margin of the hemisphere, where it undergoes a slight bifurcation.

**SULCUS OCCIPITALIS TRANSVERSUS.**—It begins 0.5cm. behind the fissura parietooccipitalis and 1cm. from the medial margin of the hemisphere. Its lateral extremity anastomoses with the sulcus occipitalis lateralis, giving rise to a stellate sulcus-complex at the junction of the occipital and temporal lobes.

**SULCI OCCIPITALES SUPERIORES.**—There is only one such sulcus. It is situated behind the sulcus occipitalis transversus. Slightly curved, it runs parallel to it from below upward to the margin of the hemisphere, and then turns and runs on the medial surface of the lobus occipitalis, delimiting a peripheral gyrus of the cuneus. Parallel to it, and separating the apical from the middle part of the cuneus on the medial surface, is a second short sulcus.

**SULCI OCCIPITALES LATERALES.**—Opposite the polus occipitalis and running parallel to the margin of the hemisphere at a distance of 1cm. from it, is a sulcus 3cm. long. Only the medial one-fourth of this is above the level of the fissura calcarina of the medial surface. The rest is opposite the gyrus lingualis. Anastomosing with this sulcus near its upper (medial) extremity is a sulcus 3.3cm. long. At the junction of its medial and anterior thirds it forms a superficial anastomosis with the sulcus occipitalis transversus, though a deep occipito-parietal annectant gyrus prevents fusion in the depth.

**SULCI TEMPORALES TRANSVERSI.**—The sulcus temporalis transversus tertius and the sulcus temporalis transversus primus are well marked, but as the gyrus temporalis transversus primus and the gyrus temporalis transversus secundus are fused, the sulcus temporalis transversus secundus is absent. Opposite the area of fusion there is a sulcus on the external surface of the gyrus temporalis superior which passes downward on the inferior surface of the gyrus and subdivides this as far as the bottom of the sulcus temporalis superior.

**SULCUS TEMPORALIS SUPERIOR.**—The sulcus is very deep and presents transverse gyri on both its walls. Its anterior extremity stops short of the frontal pole. It is not bifurcated, nor is there any transversal sulcus in front of it. At the point where the sulcus turns upward behind, it is interrupted by a superficial annectant gyrus temporalis medio-superior. Behind this the sulcus passes upward and a little backward into the gyrus angularis to within 1cm. of the sulcus interparietalis. The sulcus intermedius primus between the gyrus angularis and the gyrus supramarginalis makes a superficial anastomosis below with the anterior part of the sulcus temporalis superior. The anterior portion of the gyrus angularis is cut across by a sulcus

which anastomoses above with the sulcus interparietalis, below with the sulcus temporalis superior. An oblique sulcus, parallel to the ramus posterior ascendens and the ramus posterior descendens of the fissure of Sylvius, divides the gyrus supramarginalis into a medial and a lateral half. This sulcus anastomoses behind with the sulcus intermedius primus of Jensen.

**SULCUS TEMPORALIS MEDIUS.**—It begins in a slight bifurcation 1 cm. behind the polus temporalis and extends backward 3.8 cm., where it is interrupted by a superficial annectant gyrus temporalis medio-inferior. Behind this gyrus it begins again in a basal cross-piece nearly 3 cm. long, which cuts almost through the gyrus temporalis superior; less deeply into the gyrus temporalis medius. It then passes backward to the notch on the margin of the hemisphere formerly called the incisura præoccipitalis, and turns upward and backward for a distance of 4 cm., terminating in an H-shaped sulcus complex in the gyrus parietalis inferior posterior. Between its posterior extremity and the posterior extremity of the sulcus frontalis superior, passing down from the sulcus interparietalis between the gyrus angularis and the gyrus parietalis posterior inferior, is a well-marked sulcus intermedius secundus.

**SULCUS TEMPORALIS INFERIOR.**—The main portion of this sulcus begins about 2 cm. below and behind the polus temporalis and passes in a curved direction (concavity medialward) backward, to end in a cross-piece 2 cm. long. In front of it, between it and the anterior extremity of the lobus temporalis, is a curved transversal "compensatory" sulcus, 1.5 cm. long. Running forward to the margin of the poles from near the middle of this is a sagittal ramus 0.8 cm. in length. Behind, the sulcus is represented by a short furrow passing backward for less than 0.2 cm. behind the medial extremity of the so-called incisura præoccipitalis. The gyrus between this and the sulcus occipitalis lateralis is accordingly assumed to be the continuation backward of the gyrus temporalis inferior.

A transverse sulcus connects the fissura rhinica with the sulcus temporalis inferior and separates the incurved extremity of the gyrus temporalis inferior (in front) from the anterior extremity of the gyrus fusiformis (behind).

**FISSURA COLLATERALIS.**—Its anterior extremity is separated from the fissura rhinica by a broad gyrus rhinencephalo-fusiformis. The sulcus is deep and overhung by the gyrus fusiformis. It passes backward in a marked curve with convexity medialward. It anastomoses behind with the sulcus sagittalis gyri lingualis which bisects the gyrus lingualis. The inferior margin of the gyrus lingualis is delimited by part of an H-shaped sulcus complex. The anterior superior limb of this H runs forward into the gyrus fusiformis and subdivides its posterior portion into two sagittal gyri. The more medial of these connects with the inferior portion of the gyrus lingualis, thus forming a gyrus fusiformo-lingualis. Lateralward from the H-shaped sulcus complex the gyrus lingualis is connected with the gyrus temporalis inferior by a gyrus temporo-lingualis. The fissura collateralis is separated from the truncus of the fissura calcarina by a broad gyrus rhinencephalo-lingualis.

**SULCI INSULÆ.**—The sulcus centralis insulæ of Guldberg divides the insula into lobulus insulæ anterior and the lobulus insulæ posterior. The gyrus centralis posterior primus, gyrus centralis posterior secundus of the posterior lobulus are fused into a single mass, showing three short superficial sulci on the surface. The lobulus anterior insulæ is divided into four radiating gyri by means of three rather shallow sulci.

#### HEMISPHERIUM SINISTRUM

(Figs. 13, 15, 16, 17 and 19)

#### FISSURA CEREBRI LATERALIS [SYLVII]:

**Truncus.**—The polus temporalis does not project beyond the pars orbitalis of the gyrus frontalis inferior, except at its posterior border; the extremity of the polus is more than 1 cm. distant from the sulcus transversus.

*Fissura propria*.—This forms an angle of  $13^{\circ}$  with the longest antero-posterior diameter of the hemisphere. The distance from the ramus anterior horizontalis to the ramus posterior ascendens is 7.2 cm. The ramus posterior ascendens goes off at an angle of  $68^{\circ}$  and is 1.8 cm. long. The ramus posterior descendens measures 1.6 cm. in length. There appears to be only one anterior sulcus, the ramus anterior ascendens, but on close examination a rudimentary ramus anterior horizontalis indicating the limits of the pars triangularis can be made out, cutting only a few mm. into the cerebral substance.

SULCUS CENTRALIS [ROLANDI].—The medial extremity fails to reach by 0.5 cm. the junction of the facies convexa with the facies medialis, notwithstanding the fact that the sulcus cinguli cuts into the facies convexa for a distance of 1.2 cm. The lateral extremity is continuous with the sulcus subcentralis anterior, though there is a low annectant gyrus in the depth separating the two. The anterior subcentral sulcus appears to connect the sulcus centralis with the Sylvian fissure, but on pulling aside the margin of the latter it is seen that the subcentral sulcus terminates in the substance of the inferior surface of the operculum. The genu inferior and the genu superior are both well marked.

Relative length of sulcus centralis	- - - - -	6.7 cm.
Absolute length of sulcus centralis	- - - - -	8.6 cm.
Inclination (measured according to Eberstaller) <sup>5</sup>	- - - - -	$63^{\circ}$
Distance in a straight line of the medial end of the sulcus from the frontal pole	- - - - -	114 mm.
Ditto from the occipital pole	- - - - -	83 mm.
Distance in a straight line of the lateral end from the frontal pole	- - - - -	93 mm.
Ditto from the occipital pole	- - - - -	112 mm.

#### SULCUS PRÆCENTRALIS:

1. *Pars inferior (sulcus præcentralis inferior of Eberstaller)*.—This is only superficially connected with the pars superior, an annectant gyrus passing from the medial portion of the base of the gyrus frontalis medius to the gyrus centralis anterior, being depressed below the surface by a shallow sulcus only. The lateral extremity of the sulcus is 0.8 mm. from the margin of the Sylvian fissure, there being no separating sulcus diagonalis on this side. The medial extremity passes in front of the lateral extremity of the pars superior. There is a typical anterior sagittal ramus (ramus horizontalis of Cunningham) passing forward and a little medialward into the substance of the gyrus frontalis medius and terminating in a transversal sulcus 0.8 mm. long. The lateral portion of the foot of the gyrus frontalis medius is connected with the foot of the gyrus frontalis inferior by an annectant gyrus 0.4 cm. in thickness. The latter prevents the anastomosis of the sulcus frontalis inferior with the sulcus præcentralis inferior. The posterior extremity of the sulcus frontalis inferior almost bisects the angle formed by the sulcus præcentralis inferior with its ramus sagittalis inferior.

2. *Pars superior (sulcus præcentralis superior of Eberstaller)*.—This is an irregular sulcus, 3 cm. in length. Its lateral extremity has a shallow sagittal base 1 cm. in length. Its medial extremity passes medialward and a little forward, and, shortly before terminating, bends sharply backward into the gyrus centralis anterior to, and 1 cm. from, the medial margin of the hemisphere. The sulcus frontalis superior is continuous with the medial extremity of this sulcus. One and one-half cm. lateralward from this anastomosis a ramus sagittalis posterior passes backward into the gyrus centralis anterior, causing a deflection of that gyrus and giving rise to a knee in the sulcus centralis.

3. *Pars medialis (sulcus præcentralis medialis of Eberstaller)*.—This is represented by a short sulcus in the gyrus centralis anterior between the medial extremity of the pars superior

<sup>5</sup> The deformation of the hemisphere in hardening makes this measurement of doubtful value.



of the sulcus præcentralis and the sulcus centralis. Its medial extremity begins 2 mm. from the medial margin. The sulcus has a transversal limb extending directly lateralward for a distance of 1.5 cm. It then turns at a right angle and runs sagittally backward for 1 cm. to terminate within 0.4 cm. of the pars superior.

**SULCUS FRONTALIS INFERIOR.**—The sulcus is continuous superficially. It is prevented from uniting with the pars inferior of the sulcus præcentralis by a superficial annectant gyrus extending between the pars opercularis of the gyrus frontalis inferior, and the lateral portion of the foot of the gyrus frontalis medius. It begins behind, close to the angle formed by the pars superior of the sulcus præcentralis with its ramus sagittalis anterior, passes forward and a little lateralward for a distance of 3.5 cm., and ends in the middle of an obliquely transverse terminal sulcus 3 cm. long. The medial limb of this terminal cross-piece cuts into the gyrus frontalis medius to within 0.7 cm. of the sulcus frontalis medius, while the lateral limb cuts into the pars triangularis to within 0.5 cm. of the rudimentary ramus ascendens horizontalis of the fissure of Sylvius. Cutting into the gyrus frontalis medialis from about the middle of the sagittal portion of the sulcus frontalis inferior is a short medially-directed ramus. Eberstaller's *vordere Tiefenwindung* is distinctly visible in the depth in front of the latter.

**SULCUS FRONTALIS SUPERIOR.**—The sulcus anastomoses behind with the upper part of the pars superior of the sulcus præcentralis, though they are partially separated by a deep annectant gyrus. The sulcus at its posterior extremity is only 1.5 cm. from the medial margin of the hemisphere. It is not prolonged behind the sulcus præcentralis into the gyrus centralis anterior. It passes forward and a little lateralward (instead of, as usual, medialward) and forms in front a superficial anastomosis with the sulcus frontalis medius, an annectant gyrus making the communication a very shallow one. The sulcus is not interrupted. It presents incisures into the gyrus frontalis superior above and the gyrus frontalis medius below. In front of the anastomosis with the sulcus frontalis medius is a short isolated obliquely sagittal sulcus, and in front of this again a transversal sulcus 2.5 cm. long on the facies convexa, and extending for 1 cm. upon the facies medialis. These two isolated sulci, although behind the sulcus frontomarginalis, may, perhaps, be regarded as interrupted segments of the anterior extremity of the sulcus frontalis superior.

**SULCUS FRONTALIS MEDIUS.**—The sulcus on this side is very irregular. One cm. in front of the anterior termination of the ramus anterior of the inferior portion of the sulcus præcentralis is a transversal sulcus 3 cm. long, shallow, and unconnected with other sulci. One and one-half cm. farther forward the sulcus frontalis medius proper begins as a transversal basal piece. The medial extremity of this forms a superficial anastomosis with the sulcus frontalis superior. Passing forward from the middle of this basal sulcus a sagittal sulcus extends 1.5 cm. forward, and then bifurcates into the two limbs of the sulcus frontomarginalis of Wernicke.

**SULCUS CINGULI.**—It consists of two pieces, the pars posterior and the pars intermedius being continuous, the pars anterior being separated from the rest of the sulcus. The pars anterior begins in its usual position between the genu corporis callosi and extends forward and then upward, cutting through the medial surface of the frontal lobe to reach the margin of the hemisphere about 4 cm. behind the frontal lobe. The pars intermedius commences about 1 cm. in front of the anterior extremity of the corpus callosum and in the plane of the superior surface of that structure. It curves backward approximately parallel to the corpus callosum and, becoming continuous with the deep pars posterior, passes up behind the lobulus paracentralis to the margin of the hemisphere, and even extends for a distance of 1.3 cm. upon the convex surface.

**SULCUS OLFACTORIUS.**—One cm. deep, 4.4 cm. long. It passes forward and slightly medialward, but fails to reach the medial margin by 0.4 cm. No anastomoses.

**SULCUS ORBITALIS.**—It is much more irregular than on the right side. The sulcus orbitalis transversus is marked and anastomoses with the ramus lateralis and ramus intermedius. The ramus medialis is shallow and does not communicate with the sulcus orbitalis transversus, nor with the ramus intermedius.

**ACCESSORY SULCI OF THE LOBUS FRONTALIS:**

1. *Sulcus diagonalis operculi.*—Absent on this side, or possibly represented by the lower part of the sulcus præcentralis inferior.

2. *Sulcus radiatus.*—Not represented, though some might look upon the transversal termination of the sulcus frontalis inferior as representing the sulcus radiatus.

3. *Sulcus frontomarginalis* (Wernicke).—The lateral segment ( $fm_1$ ) is absent. The middle and medial segments ( $fm_2$ ,  $fm_3$ ) are present and appear as limbs of bifurcation of the anterior extremity of the sulcus frontalis medius. The medial limb is directly continuous in the depth with the sulcus frontalis medius, but the middle portion is partially separated by a deep annectant gyrus. In front of  $fm_2$ ,  $fm_3$  is a transverse sulcus situated exactly on the orbital margin. It may be regarded as a doubling of  $fm_3$ .

4. *Sulcus frontalis medialis and other accessory sulci of the gyrus frontalis superior.*—There is no continuous sagittal sulcus on this side. In the posterior part of the gyrus frontalis superior is an oblique curve. Farther forward are two transversal curves cutting almost entirely across the gyrus. Still more anteriorly is an oblique curve 2.5 cm. long, passing from behind and near the medial margin forward and lateralward. About half-way between this and the medial limb of the sulcus frontomarginalis is a transversal sulcus extending 2.5 cm. upon the facies convexa, and 1 cm. upon the facies medialis. The medial surface of the gyrus frontalis superior, aside from the sulcirostrales, is unusually deeply and multiply fissured.

5. *Sulcus rostralis.*—Deep and well marked; begins below the knee of the corpus callosum, runs parallel to the pars anterior of the sulcus cinguli, and terminates in a Y-shaped bifurcation. The posterior limb of the Y anastomoses with a short ramus given off by the pars anterior sulci cinguli near its extremity.

6. *Sulcus rostralis inferior.*—Three cm. long; deep; well marked.

7. *Sulcus olfactorius transversus.*—Well developed; 1.5 cm. long; situated entirely on the facies orbitalis.

**SULCUS PARACENTRALIS.**—Above the pars posterior of the sulcus cinguli is a sulcus 3.5 cm. long dividing the lobulus paracentralis into an upper and lower portion. At the anterior extremity of this sulcus a vertical ramus passes up to the margin of the hemisphere and extends for a distance of 0.4 mm. upon the facies convexa. The posterior extremity of the sulcus paracentralis is bifurcated.

**SULCUS INTERPARIETALIS:**

1. *Sulcus postcentralis.*—The sulcus is subdivided into a shorter superior and a longer inferior portion. The pars superior begins on the medial margin of the hemisphere, curves lateralward and then forward around the incisura sulci cinguli, and terminates close to the sulcus centralis. The pars inferior is an obliquely transversal sulcus, 2.5 cm. long, bifurcated at both extremities. It forms no anastomoses. It is separated from the pars superior by a gyrus which connects the gyrus centralis posterior with the lobulus parietalis superior. It is separated from a deep sulcus subcentralis posterior by a gyrus which connects the gyrus centralis posterior with the lobulus parietalis inferior.

2. *Sulcus interparietalis proprius.*—It begins 0.7 cm. behind the sulcus postcentralis inferior, being separated from it by a gyrus connecting the lobulus parietalis superior with the lobulus parietalis inferior. It passes obliquely backward and medialward to anastomose with

the sulcus paroccipitalis behind, and with the sulcus parietalis superior medially. In the depth it is partly separated from each by annectant gyri.

3. *Sulcus paroccipitalis of Wilder*.—The sulcus forms a U embracing the portion of the fissura parietooccipitalis which cuts into the facies convexa. The posterior limb of the U, after giving off a short lateral ramus, passes medialward and backward almost to the edge of the hemisphere. It does not anastomose with the sulcus occipitalis transversus.

*SULCUS PARIETALIS SUPERIOR*.—An S-shaped sulcus on the facies convexa situated between the pars superior of the sulcus præcentralis in front and the fissura parietooccipitalis behind. Its medial extremity begins at the medial margin of the hemisphere just in front of the sulcus præcuneus, being separated from the latter by an annectant gyrus. Its lateral extremity forms a superficial anastomosis with the sulcus interparietalis proprius. There is a shallow, short oblique groove in the cortex between the sulcus præcentralis superior and the sulcus parietalis superior (in the gyrus arcuatus medius), and a second shallow groove between the sulcus parietalis superior and the fissura parietooccipitalis (in the gyrus arcuatus posterior).

*SULCUS SUBPARIETALIS*.—Its anterior extremity is separated from the sulcus cinguli by a gyrus connecting the gyrus fornicatus with the præcuneus. Its posterior extremity is separated from the truncus of the fissura calcarina by a gyrus 1 cm. broad connecting the posterior inferior angle of the præcuneus with the gyrus cinguli.

*SULCUS PRÆCUNEI*.—A deep sulcus, running upward and backward from the middle of the sulcus subparietalis, bisects the præcuneus; its upper extremity terminates on the medial margin of the hemisphere just behind the beginning of the sulcus parietalis superior. The anterior and posterior halves of the præcuneus are in turn bisected by irregular vertical sulci which anastomose below with the sulcus subparietalis, terminating above in the substance of the præcuneus short of the margin of the hemisphere. The anterior vertical ramus is bifurcated at its upper extremity.

*FISSURA PARIETOOCIPITALIS*.—Distance from the medial margin of the hemisphere to point of junction with the truncus fissuræ calcarinæ, 4 cm. The fissure extends 1.7 cm. upon the facies convexa and ends without bifurcation. A typical gyrus cunei of Ecker exists. The superficies posterior lobi parietalis is very simple. There is a marked gyrus cuneo-præcuneus at about the junction of the lower and middle thirds. Above this the surface is concave and slightly irregular. There is no lobulus parietooccipitalis.

*FISSURA CALCARINA*.—Length of truncus, 1.9 cm. Length of fissura propria, 3.8 cm. The fissura is separated from the fissura parietooccipitalis in the depth by an annectant gyrus which extends from the cuneus to the gyrus cinguli. The fissura propria follows a sinuous course to the margin of the hemisphere and extends for a distance of 1.2 cm. upon the facies convexa. A sulcus sagittalis inferior cunei (Retzius) passes through the lower part of the cuneus running approximately parallel to the fissura calcarina. It extends also for a distance of 1.1 cm. upon the facies convexa.

*SULCUS OCCIPITALIS TRANSVERSUS*.—This is a deep sulcus, entirely separated above from the end of the sulcus paroccipitalis by an annectant parietooccipital gyrus 0.6 cm. broad. The course of the sulcus is sinuous. It is very convex forward below its middle. Length, 4 cm. It ends below about 1 cm. above the lower margin of the facies convexa. It forms the two posterior limbs of an X-shaped sulcus complex situated at the posterior extremity of the lobus parietalis.

*SULCI OCCIPITALES SUPERIORES*.—There is only one such sulcus. It is 2 cm. long, begins 0.5 cm. below the posterior curve of the sulcus paroccipitalis, runs nearly parallel to the upper part of the sulcus occipitalis transversus, and terminates 0.4 cm. above the uppermost sulcus occipitalis lateralis. About its middle it gives off a posterior ramus less than 0.5 cm. long.

**SULCI OCCIPITALES LATERALES.**—Of these there are two connected by a cross-piece and giving rise to an H-shaped sulcus complex. The upper of these begins 0.6 behind the sulcus occipitalis transversus, and ends behind near the margin of the hemisphere about midway between the sulcus sagittalis cunei and the fissura calcarina. The lower one ends behind below the fissura calcarina on the facies convexa, close to the polus occipitalis.

**SULCI TEMPORALES TRANSVERSI OF HESCHL.**—The sulcus temporalis transversus tertius separates the gyrus temporalis transversus secundus from the gyrus temporalis transversus tertius; it is a deep, well-marked groove which reaches the free surface of the gyrus temporalis superior and cuts through it into the sulcus temporalis superior, just in front of a well-marked superficial gyrus temporalis medio-superior secundus which exists on this side. The sulcus temporalis transversus secundus is marked only in its posterior part. The sulcus temporalis transversus primus is a very shallow groove separating the gyrus temporalis transversus primus from the insular portion of the surface.

**SULCUS TEMPORALIS SUPERIOR.**—It begins behind the polus temporalis, an obliquely placed "compensatory" sulcus intervening. One cm. behind its anterior extremity a sulcus goes off from it backward and downward to the sulcus temporalis medius. The sulcus temporalis superior is continued backward to about the junction of its anterior and middle thirds, where it is interrupted by a superficial gyrus temporalis medio-superior secundus. Behind this gyrus the sulcus begins again in the middle of a long curved transversal sulcus, the extremities of which cut into the gyrus temporalis superior and the gyrus temporalis medius respectively. The sulcus itself passes backward, curves around the projection of the gyrus temporalis superior, caused by the ramus posterior descendens of the Sylvian fissure, and then turns upward to end 0.6 cm. from the sulcus interparietalis, midway between the sulcus intermedius primus and the sulcus intermedius secundus of Jensen. At the point where the sulcus temporalis superior turns upward it anastomoses freely with the sulcus which runs backward into the lobulus parietalis posterior inferior and communicates behind with the anterior superior limb of the X-shaped sulcus complex mentioned in connection with the sulcus occipitalis transversus.

**SULCUS TEMPORALIS MEDIUS.**—It is manifoldly interrupted. It begins as a curved obliquely placed sulcus 2 cm. long, situated in front of the anterior extremity of the sulcus temporalis inferior, the segment looking like a "compensatory" sulcus for the latter. A little farther back is a shallow curved sulcus, 1.5 cm. long, connected with the oblique sulcus which anastomoses with the anterior portion of the sulcus temporalis superior. Farther back are two long, curved depressions, both of which cut deep into the gyrus temporalis medius, the anterior one cutting deep into the gyrus temporalis inferior. The posterior extremity of the more posterior of the two anastomoses with the transversal sulcus, which, in turn, forms a superior superficial anastomosis with the sulcus temporalis inferior. A broad gyrus temporalis medio-inferior now interrupts the sulcus, which begins again behind it as an obliquely transversal furrow from the middle of which the sagittal sulcus passes backward and then curves upward and backward to run toward the lobulus parietalis posterior inferior. The sulcus just above this, terminating in the lobulus parietalis posterior inferior, may be considered, if desired, as the most posterior segment of this sulcus temporalis medius.

**SULCUS TEMPORALIS INFERIOR.**—It begins 2 cm. behind the polus temporalis and runs backward for 5.5 cm. It is then interrupted by a gyrus connecting the gyrus temporalis inferior with the gyrus fusiformis. Behind this annectant gyrus, a sagittal continuation of the sulcus forms the cross-piece of an H-shaped sulcus complex, the side bars of the H being transversal sulci which pass down from the sulcus temporalis medius, across the gyrus temporalis inferior into the gyrus fusiformis. Another annectant gyrus between the gyrus temporalis inferior and the gyrus fusiformis again interrupts the sulcus. Behind this the continuation of the sulcus forms

the top piece to a  $\pi$ -shaped terminal sulcus complex. Between its posterior extremity and the lower extremity of the sulcus occipitalis transversus a third gyrus connects the gyrus temporalis inferior with the gyrus fusiformis.

**FISSURA COLLATERALIS.**—This is shallower than on the right side, is separated in front from the gyrus hippocampi by a long, narrow gyrus which runs from the incurved *polus temporalis* to the middle of the gyrus hippocampi. Behind, it ends near the posterior extremity of the hemisphere, in an obliquely transversal cross-piece, the medial limb of which goes between the two vertical pieces of the  $\pi$ -shaped sulcus complex at the posterior extremity of the sulcus temporalis inferior. The gyrus lingualis is subdivided by a sagittal sulcus which anastomoses in front with the fissura collateralis below the annectant gyrus rhinencephalo-lingualis.

**SULCI INSULÆ.**—Guldberg's central sulcus of the island separates the posterior lobe with its two gyri from the anterior lobe with its four gyri.

#### THE CEREBELLUM

(Figs. 21, 22, 23, 24, and 26)

The cerebellum looks a little small. It has, however, been distorted in the hardening process. The actual measurements are as follows:

Length (anteroposterior), in the middle	- - - - -	4.1 cm.
Right side (maximal)	- - - - -	6.7 cm.
Left side (maximal)	- - - - -	6.6 cm.
Greatest transverse diameter	- - - - -	9.2 cm.
Greatest height or thickness	- - - - -	4.2 cm.

The distortion has flattened the vertical and increased the anteroposterior diameter. The *incisura cerebelli posterior* is deep.

**Vermis.**—The *lingula cerebelli* has been torn in separating the cerebellum from the rest of the brain stem. It is well developed, is not atrophic or rudimentary, and is a *lingula simplex*, not duplex. The *vincula lingulæ cerebelli* are symmetrical and not separated from the *lingula* by notches. The two sides of the posterior free surface of the *lingula* are symmetrical. The anterior surface is firmly fused posteriorly with the *velum medullare anterius*.

**Lobulus centralis.**—This is well developed. It lies almost entirely on the anterior free surface of the cerebellum, but its most superior portion is visible from the *facies superior* and thus enters to a slight extent into the formation of this surface. On its anterior surface eight *Randwülste* are present, separated from one another by depressions. These *Randwülste* run in general parallel to one another and vary only slightly in thickness. The upper three *Randwülste* are marked off from the *ala lobuli centralis* on each side by deep indentations; below these three, however, the junction is grooved, but there is no incisure. The delimitation is more sharply marked off on the left than on the right side.

The posterior surface of the *lobulus centralis* is slightly concave. It presents seven *Randwülste* of approximately equal width. There are no incisures between the *Randwülste* of the central lobule and its *alæ*.

The apex of the central lobule is rather blunt.

The dimensions of the *lobulus centralis* are as follows:

##### A. Of *facies anterior*:

1. From above downward (apex to base)—in the middle - - - 17 mm.  
(Stilling's normal measurements vary between 10 and 20 mm.)
2. From right to left—
 

Above	- - - - -	9 mm.
	(Stilling's normal, 4-10 mm.)	
Middle	- - - - -	9.5 mm.
	(Stilling's normal, 6-10 mm.)	

## B. Thickness:

Just beneath apex	- - - - -	2 mm.
In the middle	- - - - -	5 mm.
At the base	- - - - -	4 mm.

The alæ lobuli centralis present on both sides the typical double concavity on the anterior free surface corresponding to the brachium conjunctivum and the brachium pontis. The anterior free surface of each ala is subdivided by sulci into numerous *Randwülste* more markedly than in most of Stilling's cases; the configuration is somewhat different on the two sides. The right ala presents seven *Randwülste*, the longest being the fifth, which measures 15 mm.; the upper two are the shortest. The sulci do not extend all the way to the posterolateral margin. There is no fusion with the brachium conjunctivum, the two being separated by the pia mater. The left ala also presents seven *Randwülste*. There is a tendency to a radial arrangement of the sulci from a center situated near the lateral apex. An area which is not subdivided exists near this apex. The posterior free surface of the alæ presents well-marked *Randwülste* and sulci; on the left side this surface is fused with the lobus intermedius. The dimensions of the anterior surface of the ala lobuli centralis are as follows:

## From above downward:

a) Next the lobulus centralis, right side	- - - - -	12 mm.
left side	- - - - -	11 mm.
(Stilling's average, 11-12 mm.)		
b) In the middle, right side	- - - - -	10 mm.
left side	- - - - -	10 mm.
(Stilling's average, 7-8 mm.)		
c) Behind middle of brachium pontis, right side	- - - - -	7 mm.
left side	- - - - -	10 mm.
(Stilling's average, 4-5 mm.)		

## Width:

a) Upper margin, right side	- - - - -	18 mm.
left side	- - - - -	22 mm.
(Stilling's average, 16-22 mm.)		
b) Lower margin, right side	- - - - -	16 mm.
left side	- - - - -	18 mm.
(Stilling's average, 14-20 mm.)		

In general, then, it may be stated that the lobulus centralis and its alæ are well developed and the asymmetry is within normal limits.

*Monticulus*.—The monticulus on examination is found to be developed in both culmen and declive. There is a high lobus intermedius between the lobulus centralis and the first portion of the culmen. Its apex does not, however, reach the surface of the vermis superior. The general configuration of the culmen, when distortion has been discounted, corresponds very closely to that depicted in Stilling's Fig. 122. The declive is also typical, though the sagittal section (Fig. 26) has, in this region, deviated somewhat from the median plane, so that the posterior part of the section passes through the edge of the hemisphere and not through the posterior part of the worm.

*Other parts of vermis*.—The tuber, pyramis, uvula, and nodulus of the vermis inferior are present in the gross specimen in normal arrangement.

*Hemisphaeria cerebelli*.—The hemispheres show no marked microscopic alterations.

*Lobulus quadrangularis*.—Counts of the *Randwülste* give the following results:

## Upper surface:

## Right side—

1. Near vermis	- - - - -	21
	(Stilling's normal, 17-19)	
2. Near pons	- - - - -	14
	(Stilling's normal, 12-14)	
3. In middle, between vermis and pons	- - - - -	20
	(Stilling's normal, 22-26)	

## Left side—

1. Near vermis	- - - - -	14
	(Stilling.)	
2. Near pons	- - - - -	15
3. In middle, between vermis and pons	- - - - -	23

The six walls (*Wände*) correspond to the six *Wände* of the monticulus. The first wall is doubled on the left side; the second, on the right side. The fourth wall is but slightly developed, being represented by a projection in the depth, between the third and fifth walls.

The dimensions of the lobulus quadrangularis are as follows on the upper surface:

1. In sagittal direction next to monticulus, left	- - - - -	4.3 cm.
right	- - - - -	4.6 cm.
	(Stilling, 33-40 mm.)	
Next to brachium pontis, left	- - - - -	2.3 cm.
right	- - - - -	2.4 cm.
	(Stilling, 18-20 mm.)	
2. In transversal direction on anterior margin, left	- - - - -	2.0 cm.
right	- - - - -	2.4 cm.
	(Stilling, 28-35 mm.)	
On posterior margin, left	- - - - -	5.8 cm.
right	- - - - -	5.7 cm.
	(Stilling, 65-72 mm.)	

*Lobulus semilunaris superior.*— On the right side the number of *Randwülste* on the surface near the incisura cerebelli posterior is five; on the left side, seven. On the right side, on the surface in the middle, there are thirteen *Randwülste*; on the left side, fourteen.

The maximal sagittal measurement of the upper surface on the right side is 1.2 cm.; on the left side, 1.8 cm. The curved measurement transversally along the posterior margin is on the right side 7.5 cm.; on the left side, 6.5 cm.

*Lobulus semilunaris inferior:*

Number of <i>Randwülste</i> near vermis, right	- - - - -	8
left	- - - - -	10
“ “ “ lateral margin, right	- - - - -	4
left	- - - - -	3
“ “ “ in middle, right	- - - - -	8
left	- - - - -	9
Maximal sagittal measurement, right	- - - - -	1.7 cm.
left	- - - - -	2.2 cm.
Maximal length of posterior margin, right	- - - - -	7.4 cm.
left	- - - - -	7.0 cm.

Number of <i>Randwulste</i> near vermis, right	-	-	-	-	-	-	5
left	-	-	-	-	-	-	3
“ “ “ lateral margin, right	-	-	-	-	-	-	4
left	-	-	-	-	-	-	3
Maximal sagittal measurement, right	-	-	-	-	-	-	1.1 cm.
left	-	-	-	-	-	-	0.9 cm.
Maximal sagittal measurement (curved), right	-	-	-	-	-	-	6.9 cm.
left	-	-	-	-	-	-	6.0 cm.

Number of <i>Randwülste</i> in middle of inferior surface, right	-	-	13
left	-	-	12
Sagittal measurement, in middle, right	-	-	3 cm.
left	-	-	2.9 cm.
Length of posterior margin, right	-	-	5 cm.
left	-	-	4.9 cm.

Number of <i>Randwülste</i> on inferior surface, right	-	-	-	-	5
left	-	-	-	-	(injured)
Dimensions of inferior surface, right	-	-	-	-	1.3×0.7 cm.
left	-	-	-	-	1.2×0.9 cm.

On the right side the lateral mass measures 2 cm. in length; the medial mass the same. There are eight *Randwülste* on the medial surface of the medial mass; and seven on the lateral surface of the lateral mass.

(Figs. 45, 46 and 35)

There is a noticeable increase in the number of Unna's plasma cells in the walls of the large blood-vessels within the substance of the cord, as well as in the vessels of the glia. This is true of all portions of the cord, pars lumbalis, thoracalis, et cervicalis.

[illegible][illegible]



The anterior and posterior roots of the spinal nerves show no alteration, except possibly a slight increase in the endoneurium.

The pia mater is a little thickened.

Thickness of pia over funiculus lateralis	- - -	0.076 to 0.155 mm.
Thickness of pia over funiculus posterior	- - -	0.048 to 0.081 mm.
Thickness of pia over funiculus anterior	- - -	0.037 to 0.087 mm.

The subpial layer of neuroglia (*Gliahülle* of the Germans) is much thicker in the lumbar region than normal. The average thickness over the funiculus anterior is 0.48 mm. There are two triangular indentations in the anterior part of the cord where this glial layer measures 0.261 mm. and 0.243 mm. in depth.

Thickness of subpial layer of glia over fasciculus Lissaueri	-	0.152 mm.
Thickness of subpial layer of glia over funiculus posterior (lateral part)	- - - - -	0.017 mm.
Thickness of subpial layer of glia over funiculus posterior (near median septum)	- - - - -	0.094 mm.

*Study of sections stained with Weigert's myelin sheath stain or with iron hæmatoxylin stain of chromicized tissues.*—The fasciculi stain, on the whole, normally. There are no degenerated areas. An exception is to be made for the middle root zone (Flechsig) of the funiculus posterior, which is lighter than the rest of the funiculus, and the glia is more abundant than normal in this lightened area. In view of the fact that the nucleus dorsalis and the direct cerebellar tract higher up are degenerated, it is interesting to note that it is this middle root zone of Flechsig in the lumbar cord which contains those posterior root fibers which run in to end in the gray matter of the nucleus dorsalis at a higher level. Aside from the alteration in this middle root zone, the dorsal funiculi show no change. Lissauer's fasciculus is well developed. The pyramidal tracts and fasciculi proprii of the funiculus lateralis look normal. No alteration can be made out in the funiculus anterior. The fine medullated fibers (terminals and collaterals) which pass through the substantia gelatinosa appear normal in number and distribution. The anterior and posterior white commissures show no change.

*Sections stained by Nissl's method, in thionin, and in toluidin blue.*—The anterior horn cells are present in nearly normal number, though they may be slightly reduced. No change in size, shape, or distribution is discoverable. The internal morphology of these cells appears to be quite normal. The nuclei are centrally placed, and the nucleoli stain intensely. The stichochrome arrangement of the tigroid masses is well preserved in the dendrites and also in the perikaryons. Abundant masses of lipochrome can be seen in many of the anterior horn cells.

**PARS THORACALIS.**—The cord has been a little flattened on removal. The dimensions are:

Anterior posterior diameter	- - - - -	0.5 mm.
Transverse diameter	- - - - -	0.9 mm.
Average diameter in the two directions	- - - - -	0.7 mm.

Professor Donaldson's measurements of a cross-section show an area of white matter of 6.955 sq. cm. and an area of gray matter of 0.673 sq. cm. This is a ratio of 1:13,<sup>6</sup> which is clearly abnormal.

The anterior and posterior roots of the spinal nerves show no changes.

The subpial layer of neuroglia measures as follows:

<sup>6</sup>Cf. Donaldson, H. H. and D. G. Davis. A description of chart showing the areas of the cross-sections of the human spinal cord at the center of each spinal nerve. *J. Comp. Neurol.*, Granville, Vol. XIII (1903), pp. 18-39.

Thickness over funiculus lateralis (direct cerebellar tract)	- - -	0.35 mm.
Thickness over funiculus anterior	- - - - -	0.26 mm.
Thickness over funiculus posterior (funiculus cuneatus)	- - -	0.35 mm.
Thickness over funiculus posterior (funiculus gracilis)	- - -	0.22 mm.

*Sections stained with Weigert's myelin sheath stain or with iron hæmatoxylin after chromicizing. (Fig. 45.)*

*Funiculus anterior.*—Nothing abnormal discernible.

*Funiculus lateralis.*—The fasciculus spinocerebellaris dorsolateralis (direct cerebellar tract) is almost entirely degenerated. Throughout the whole thoracic portion of the spinal cord a narrow stripe, situated on the dorsolateral surface of the cord, corresponding to the position of this tract, is almost devoid of medullated fibers, its place being represented only by glia. This area of degeneration does not extend farther forward, in the middle and lower portion of the thoracic cord at any rate, than a point opposite the columna intermediolateralis of the substantia grisea. It extends backward for a variable distance and usually does not reach as far behind as Lissauer's fasciculus. The internal margin of the degenerated stripe is irregular, corresponding to the admixture of the direct cerebellar tract with adjacent fiber systems (pyramidal tract). The external margin of the degenerated stripe is the periphery of the cord. Scattered medullated fibers still exist in the degenerated area. These vary in diameter from 5 to 15 microns, the majority being large fibers (the average diameter of the fibers of the direct cerebellar tract is about 8 $\mu$ ).

The maximum depth of the degenerated area measured from the surface of the cord inward is on one side 0.315 mm. and on the other 0.560 mm. The degenerated area begins, as a rule, 1 mm. anterior to Lissauer's funiculus, and extends ventralward for a distance of 2.5 mm. The area is comma-shaped, the head of the comma being directed ventralward and the tail dorsalward.

The fasciculus spinocerebellaris ventrolateralis (Gowersi) shows no recognizable alterations, at least in the middle and lower thoracic cord. The fasciculi cerebrospinales laterales look normal, as do the fasciculi laterales proprii.

*Funiculus posterior.*—A light strip near the medial septum on each side, ventrally continuous with a much less lightened and very diffuse area in the lateral portion of the fasciculus cuneatus on each side, is visible. The glia is slightly increased in amount in this area. The areas correspond to the distribution of the third system of fibers (foetus 35 cm. long) of Trepinski's developmental studies—that is, to precisely the same system of fibers as that to which belong the fibers of the middle root zone of the lumbar portion of the spinal cord. Otherwise the funiculi posteriores show no alterations.

*Sections stained by Nissl's method, in thionin, and in toluidin blue.*—Sections were taken from ten different levels of the pars thoracalis. The whole cord is flattened anteroposteriorly, possibly owing to slight injury on removal. With this flattening there is marked displacement of the posterior horns to the side. The anterior horn cells are present in normal numbers, are of the usual size, and present the typical stichochrome arrangement of their tigroid masses.

The cells of the columna intermediolateralis are a striking feature of the sections throughout the pars thoracalis, and they are present in normal numbers, are elliptical usually in shape, and measure from 7 $\mu$  to 38 $\mu$  in the long diameter and from 5 $\mu$  to 18 $\mu$  in the short diameter (Ziehen gives as the normal 12 to 15 $\mu$  in the long, and 5 to 15 $\mu$  in the short, axis). The tigroid substance in these cells is very variable. The masses are irregular in size, shape, and distribution. In many of the cells the tigroid masses are arranged at the periphery, the center of the cell being pale or staining diffusely blue. The nuclei of some of these cells are situated at one pole or one edge of the cell; they may have a crescentic shape and look shrunken.

The cells of the columna posterior include (1) the cells of the nucleus dorsalis (Clarke's column), (2) the cells of the substantia gelatinosa Rolandi, (3) the cells of the zonal layer (*cellules limitantes*), and (4) the cells of the columna posterior propria (Waldeyer's nucleus of the dorsal horn).

Of these, the cells of Clarke's column interest us most, for they have almost entirely disappeared throughout the whole length of the nucleus. In many sections not a single cell is visible; where any cells are left at all, the number does not exceed from one to three in a section (Fig. 35). The few cells remaining present no characteristic alterations when compared with the normal cells of control preparations. The tigroid masses are irregular in size and distribution; occasionally they are peripherally disposed. The nuclei may be eccentrically placed, and the nucleoli stain intensely.

An examination of the so-called Gierke's cells of the substantia gelatinosa has been made. On comparison with control preparations, they appear to be normal.

The marginal cells of the zonal layer are normal in number and appearance. I cannot detect any alteration in the triangular cells of Waldeyer's nucleus of the dorsal horn.

#### PARS CERVICALIS AND TRANSITION INTO MEDULLA:

*Sections stained by Weigert's method, and with iron hæmatoxylin.*—The degenerated area still corresponds to the situation of the direct cerebellar tract. The area is larger than in the thoracic portion of the cord, and begins nearer the dorsal horn. In sections through the junction of the spinal cord with the medulla oblongata the degenerated area is exquisitely marked out. It is approximately triangular in shape, the apex of the triangle being on the surface, behind, opposite the dorsal horn, the base in front. The two sides of the triangle are formed by the periphery of the cord and the lateral surface of the lateral pyramidal tract respectively. On one side of the cord a large bundle of pyramidal tract fibers plunges directly through the degenerated area, just after decussation: these fibers are surrounded on all sides by the pale yellow glia of that part of the cord from which fibers of the direct cerebellar tract have almost entirely disappeared (*vide* Fig. 41). The distance from the apex to the base of the triangle is 2.128 mm.; the width of the base is 0.598 mm. A few scattered healthy fibers remain in the degenerated area, as was the case in the sections through the pars thoracalis. These are chiefly fibers of very large caliber. The subpial neurologia layer over the degenerated area measures 0.113 mm. in thickness.

There is slight diffuse lightening of the area extending for 1 to 1.5 mm. in front of the main triangle of degeneration, and with the high power it is seen that the nerve fibers do not stand so closely together here as they do normally. Either a few fibers of the direct cerebellar tract have been present in this area, or we have to deal with a slight loss of fibers in the ventral spino-cerebellar tract of Gowers.

In the dorsal funiculi there is a long, narrow, slightly lightened strip close to the medial margin of the fasciculus gracilis, and a still more diffuse, slightly lightened area near the lateral middle portion of the fasciculus cuneatus. There has been an actual loss of fibers from these areas.

The fasciculi cerebrospinales contain no lightened areas in the cross-sections. This is true, also, of the various fasciculi proprii. Helweg's path is not distinguishable by any color differences from the surrounding white matter.

*Sections stained by Nissl's method, with thionin, and with toluidin blue.*—The cells of the ventral horns are present in normal number and distribution. The tigroid substance is abundant, and a large number of these cells are richly provided with masses of lipochrome. No alteration in any of the nerve-cells in this portion of the nervous system is discernible.

## MEDULLA OBLONGATA

(Figs. 38, 39, 40, and 41)

LEVEL OF DISTAL PORTION (ABOVE DECUSSATIO PYRAMIDUM AND AT THE LOWER PART OF THE DECUSSATIO LEMNISCORUM, CENTRAL CANAL STILL CLOSED):

*Sections stained with Weigert's myelin sheath stain or with iron hæmatoxylin.*—There is a triangular area of degeneration in the position of the direct cerebellar tract which is situated, at this level, just ventrolateral from the laterally deflected dorsal horn on each side. The apex of the triangle is directed medialward; the base, somewhat curved, corresponds to the periphery of the medulla. Between the apex of the triangle and the most ventral portion of the substantia gelatinosa, an area, 0.25 mm. in breadth, of undegenerated, rather fine fibers, intervenes. On the very periphery of the medulla, the area of degeneration is bounded by some medullated fibræ arcuatæ externæ.

The funiculi posteriores are normal in appearance, as are the pyramids and the bundles in the regions of the medulla other than that of the direct cerebellar tract. The spinal tract of the trigeminal nerve looks normal.

## LEVEL OF NUCLEUS NERVI HYPOGLOSSI AND NUCLEUS ALÆ CINERÆ:

*Sections stained by Weigert's method.*—The pyramids and stratum interolivare lemnisci are normal on each side. The fibræ arcuatæ internæ and fibræ arcuatæ externæ appear normal. The tractus spinalis n. trigemini and the fasciculus solitarius are well developed and unaltered.

The degenerated area corresponding to the direct cerebellar tract has disappeared at this level, apparently having turned into the corpus restiforme. The latter structure, however, appears to be evenly dark and the position of the degenerated fibers is not ascertainable. The root fibers of the nervus hypoglossus look normal. The fibræ olivocerebellares show no obvious change. No extensive alterations have been made out in the various masses of gray matter at this level—nucleus nervi hypoglossi, nucleus alæ cineræ, nucleus olivaris inferior, nuclei olivares accessorii, nucleus funiculi cuneati, and nuclei arcuati. The number of cells in the gray matter of the olive is probably smaller than normal; indeed, the size of all the structures seems somewhat diminished (*cf.* Fig. 33).

## LEVEL OF PROXIMAL EXTREMITY OF THE MEDULLA OBLONGATA AT REGION OF ENTRANCE OF N. ACUSTICUS:

*Sections stained by Weigert's preparations.*—No alterations are visible in the vestibular nerve or its nuclei.

## PONS VAROLII

## LEVEL OF BRACHIUM CONJUNCTIVUM AND VELUM MEDULLARE ANTERIUS:

The brachium conjunctivum is smaller in volume than normal; the fibers are a little thinned; the average caliber of the fibers is small; the glia is rather more abundant than normal. The fasciculus longitudinalis medialis, though rather small in volume, in transverse section stains normally. There is no degeneration of the lemniscus lateralis. The fasciculus tegmenti centralis (*centrale Haubenbahn*) is of normal size and stains well. The longitudinal fasciculi and the transverse fibers of the pons stain normally. The gray matter of the formatio reticularis in the pars dorsalis pontis and that of the nuclei pontis in the pars ventralis appear to be normal in amount and distribution.

## CEREBELLUM

## WEIGERT PREPARATIONS

## STUDY OF SAGITTAL SECTIONS THROUGH CEREBELLUM:

1. *Sagittal sections near median plane of vermis* (Fig. 26).

a) *Corpus medullare.*—The laminæ medullares look normal in Weigert preparations. In

the corpus trapezoideum and the main vertical limb of the arbor vitæ the transversely running fibers of the great anterior decussation are to be seen.

b) *Substantia corticalis*.—Except that the vermis is on the whole somewhat smaller than in the brother's brain, the appearances are very much the same. The molecular layer is separated from the granular layer by the layer of ganglion cells. The latter are very irregularly distributed, but this is true of the normal cerebellum.

c) *Nucleus fastigii*.—The area for this nucleus is present in its normal position, but the study of a number of sections shows an apparent diminution in the number of nerve-cells present in it. The nerve-cells found are smaller than normal. Glia cells are abundant. The medullated fibers extending through the brachium conjunctivum between the region of Deiter's nucleus in the medulla and the region of the nucleus of the roof are a prominent feature of the sections; they are present in apparently normal numbers.

2. *Sagittal section about  $6\frac{1}{2}$  mm. or 7 mm. lateral from the median plane*.—The section passes through the nucleus globosus and the nucleus emboliformis and through the medial portion of the hemisphærium cerebelli, thus cutting, above, the lobulus quadrangularis and the lobulus semilunaris superior, and, below, the lobulus semilunaris inferior, the lobulus gracilis, the pyramis, and the tonsilla.

a) *Substantia corticalis*.—No alterations are discernible.

b) *Nucleus emboliformis*.—In this section this nucleus is spindle-shaped, the maximum length of the spindle being 8 mm., its maximum dorsoventral diameter  $2\frac{1}{4}$  mm.; above it are three minute islands of gray matter, which belong to the most medial part of the nucleus dentatus, while below and behind it is the mushroom-like head of the nucleus globosus. Beneath it and in front of it are the fibers of the brachium conjunctivum. The section evidently passes through only the more lateral and posterior part of the nucleus emboliformis. Large numbers of large ganglion cells are present.

c) *Nucleus globosus*.—This section passes through the posterior head of the mushroom-like nucleus and through the posterior part of its stem.

Greatest vertical diameter in sections = 2 mm.

Greatest sagittal diameter in sections =  $5\frac{1}{2}$  mm.

Owing to the flattening of the cerebellum as a whole in the hardening process, it is probable that the vertical diameter is here very small owing to an increase in the transversal diameter. The nucleus globosus is separated from the under surface of the nucleus emboliformis by a well-preserved narrow band of medullated fibers. Numbers of ganglion cells are present in the nucleus.

d) *Nucleus dentatus*.—Only three small islands of ganglion cells are present; these are obviously cross-sections through the most medial projections of the dentate nucleus.

e) *Corpus medullare*.—The laminæ medullares of the different cortical areas are well developed and fuse with the central white substance. The transversely and obliquely cut fibers corresponding to the anterior and posterior decussation-commissures look normal. The fibers of the brachium conjunctivum (Stilling's processus cerebelli ad corpora quadrigemina) look a little thinner and the blood-vessels in it are dilated.

3. *Sagittal section through hemisphærium cerebelli about 14 mm. lateral from sagittal median plane of vermis*.—The section passes through the lobus quadrangularis, lobulus semilunaris superior, lobulus semilunaris inferior, lobulus gracilis, lobus biventer, and the flocculus, the lateral part of the nucleus dentatus, the fibræ semicirculares cerebelli, and the central part of the brachium pontis.

The nucleus dentatus is here growing small in size and is much convoluted. The cells in its gray matter are more numerous than in the nucleus dentatus of the brother, but less

numerous than in a normal control. Each cell contains a mass of yellowish green pigment (in toluidin blue preparations) which is probably identical with the lipochrome of anterior horn cells. I can make out no decided alterations in the corpus medullare at this plane.

4. *Sagittal sections through hemisphaerium cerebelli lateral from the nucleus dentatus.*—The corpus medullare gradually grows smaller in volume, the fibræ semicirculares less distinct as in normal preparations. No alterations other than the general diminution in volume are discernible.

#### STUDY OF FRONTAL SECTIONS THROUGH CEREBELLUM:

1. *Frontal section a few millimetres in front of apex of culmen.*—This section passes in front of all the gray nuclei of the cerebellum and shows, besides the cortical portions of the vermis and hemisphaerium, the great central mass of white matter just as it is going over into the brachium pontis. No areas of degeneration are visible.

2. *Frontal section near apex of culmen.*—The section has passed a little obliquely lateralward and backward, having caught the jagged edge of the latero-inferior portion of the anterior extremity of the nucleus dentatus. The mass of fibers corresponding to the area of the brachium conjunctivum looks thin and lightened in Weigert preparations, though it is crossed by obliquely cut strands of deeply staining fibers of large caliber. The latter accumulate near the nucleus fastigii. The latter is not well developed, and relatively few cells are to be found in it.

3. *Frontal section about 6 mm. behind highest point of culmen.*—The origin of this section is easily recognizable by the horseshoe shape presented by frontal sections of the nucleus emboliformis in this plane. Below it is a cross-section of the nucleus globosus, here represented by a large, rather triangular mass with two small gray masses lateral from it and one small gray mass just medial from it. All these lie in the hilus nuclei dentati. The only representative of the nucleus fastigii is a minute mass of gray matter near the median plane of the vermis.

The white matter within the nucleus dentatus looks pale in Weigert preparations, and the blood-vessels are dilated. This pallor is not so marked, however, as in the brain of the brother.

The great anterior decussation-commissure is well developed and stains intensely in the Weigert preparations.

4. *Frontal section through the declive and posterior extremity of the nucleus dentatus.*—Sections through this level show the most posterior portions of the nucleus dentatus imbedded in the corpus medullare. Sections through the nucleus dentatus when compared with sections from the normal cerebellum reveal a smaller number of nerve-cells.

#### NISSL PREPARATIONS

Sections were made from the cortex of the vermis and of the cerebellar hemispheres. No striking differences in appearance in the sections from the two sources could be made out, except that the Purkinje cells in the hemisphere look smaller than in the vermis. (Cf. Figs. 28 and 29.)

The *molecular layer* measures from 0.326 mm. to 0.363 mm. in thickness. It is thicker near the bases than at the summits of the folii.

The *granular layer* measures 0.4 mm. in thickness at its thickest part. It is, however, very variable in depth and near the bottom of the interlamellar sulcus may be as thin as 0.1 mm. These measurements are smaller than those given as normal by Poirier et Charpy, according to whom the molecular and granular layers are of equal thickness, each measuring 0.5 mm. in depth. Von Kölliker, however, gives as the normal thickness of the molecular layer in human beings the measurements 0.15–0.40 mm.; he does not make any statement regarding the thickness of the granular layer.

The Purkinje cells are of normal size in the vermis, but rather small in the hemisphere; they present cell bodies rich in stainable substance of Nissl, large, pale nuclei, and large, deeply

staining nucleoli. The question, "Are the Purkinje cells present in normal numbers?" is a difficult one to answer. They often lie very close together in the single row in which they are arranged, but wide gaps between Purkinje cells are very frequently met with (*vide* Fig. 29). These gaps may be as wide as 0.6 mm. Stilling's statement with regard to the distances between the individual Purkinje cells is as follows:

Die grossen Nervenzellen liegen nämlich, in mehr oder minder regelmässiger Weise, in mehr oder minder grossen Strecken von einander entfernt, und lassen also einen Zwischenraum, meist je von  $1/50'''$ , zwischen sich; oft stehen sie auch gedrängter in einer und derselben Ebene, nur durch eine Entfernung je von  $1/150'''$  getrennt; nicht selten berühren sich sogar mehrere unmittelbar. In manchen Ebenen sind sie dagegen wieder auffallend weit, bis zu  $1/12'''$ , von einander entfernt. Da man in seinen Abschnitten doch meist nur Theile der einzelnen grossen Nervenzellen (mutilirte) zu Gesicht bekommt, so übersieht man gewiss oft genug Fragmente dieser Nervenzellen, und wo eine auffallend lange Strecke ohne grosse Nervenzellen erscheint, lagen vielleicht dennoch einige, die durch die Präparation unkenntlich wurden. Immerhin aber treten in den, zwischen den einzelnen grossen Nervenzellen befindlichen, grösseren oder kleineren Zwischenräumen dennoch die einzelnen Elemente der Zellen- und Körnerschicht in unmittelbare Berührung resp. Verbindung, d. h. an zahllosen Stellen.

If he had dealt with thinner sections, he would doubtless have met with instances of greater intervals, but, even allowing for the difference in thickness of sections, it would seem not unlikely that there is in the case here described an actual diminution in the total number of Purkinje cells as compared with Stilling's findings. On comparison with control preparations, however, I am not convinced that the number of Purkinje cells is below normal. In Fig. 27 has been reproduced a section of the hemisphere of the cerebellum of a physician dead of myasthenia gravis, in which disease there is presumably no diminution in the number of Purkinje cells. The interspaces between cells are seen to be very large.

In Krohm's<sup>1</sup> case of atrophy of the cerebellum, met with in an ataxic cat, studied in Donaldson's laboratory, there was extensive atrophy of the cerebellum as a whole, with marked diminution in thickness, particularly of the molecular layer. In places the Purkinje cells had disappeared entirely, leaving open, empty spaces in their places, and the Purkinje cells in general were markedly shrunken. There seemed to be but little alteration in the granular layer or in the white medullary substance.

#### CEREBRAL CORTEX:

*Gyrus centralis anterior*.—The gray matter is 2 mm. thick on the surface, 2.5 to 3 mm. thick on the walls of the sulci. The plexiform layer measures 0.319 mm. in thickness. The various layers of pyramidal cells are present in Nissl preparations. I can make out no definite alterations in them.

*Gyrus cuneus (near fissura calcarina), Nissl preparations*.—The plexiform layer measures 0.156 mm. in depth. It contains a very few scattered nuclei, the majority being small and round and looking like ordinary glia nuclei. A few polygonal and spindle-shaped nuclei are, however, also present in this layer. The layer of small pyramidal cells and the layer of medium-sized pyramidal cells together measure 0.438 mm. in depth. The layer of large stellate cells is 0.133 mm. deep; that of the small stellate cells, 0.292 mm.; and that of the small pyramidal cells, with ascending axones, 0.119 mm. deep. The giant pyramidal cells are widely removed from one another and their cell-bodies and nuclei look normal. The layer of pyramidal cells with curved, ascending axones measures 0.199 mm. in depth and the layer of spindle-shaped cells, 0.20. The total thickness of the gray matter here is 1.6 mm. in its thinnest part.

<sup>1</sup> W. O. KROHM, "Atrophy of Cerebellum in Cat," *Journal of Nervous and Mental Diseases*, New York (October, 1892).

## RÉSUMÉ OF MORBID ANATOMY

The brains and cords of both cases are relatively small, the cords, medulla, and pons in each case looking smaller in proportion than the cerebrum.

The cerebral cortex in both cases is well fissured; indeed, if there be abnormality, it is on the side of over-fissuration rather than on that of under-fissuration. The form of the various sulci and gyri is fully described in the protocols, and a study of these reveals no deviations from the normal type which can be regarded as standing in relation to the disease from which the family suffered. The cerebral topography is, however, interesting, aside from the pathology of the case, in that it presents an opportunity for comparing the similarities and differences in the brain-surfaces of two brothers.

The cerebellum, though a little small in each case, is very typical in macroscopic configuration. The cerebellar peduncles (superior, middle, and inferior) are all relatively small; the diminution in size is not marked, however.

The spinal cord looks a little smaller than normal in each case, that of Case XVIII looking smaller than that of Case XX; and this is in accord with the actual measurements. Measurements made by Professor Donaldson show an abnormal ratio between the area of white matter and the area of gray matter in the cross-sections.

The microscopic study in both cases reveals marked degeneration in the gray and white matter of the spinal cord, medulla oblongata, and cerebellum. The degeneration is considerably more advanced in Case XVIII than in Case XX, though the difference is in degree rather than in kind. The degeneration involves in both cases chiefly nerve-cells and nerve-fibers of centripetal paths, including one system of exogenous fibers of the posterior funiculus (apparently corresponding to the third foetal system of Trepinski), the dorsal nucleus of Clarke, the direct cerebellar tract of Flechsig in the lateral funiculus and its continuation in the restiform body. In these structures the degeneration is very extensive; in addition there has been some involvement of the dentate nucleus of the cerebellum and the brachium conjunctivum, and probably also of the inferior olivary nucleus of the medulla oblongata; in the more advanced case there may be a slight diminution in the number of the anterior horn cells, and of the fibers of the nerve-roots.

Corresponding to the degeneration in the white fibers of the spinal cord, there has been an increase in the glia tissue—an increase which is relatively more pronounced in the dorsal funiculus than in the area of the lateral funiculus corresponding to the direct cerebellar tract.

Only one case from this family has been previously studied histologically, but that one, fortunately, by Dr. Adolf Meyer,<sup>2</sup> who found no circumscribed cerebellar lesion, but made out a marked diminution in the number of cells in Clarke's nucleus, a degeneration of the direct cerebellar tract, and an elective degeneration of the posterior funiculus, especially marked in the pars cervicalis, where it involved the medial

<sup>2</sup> A. MEYER, "The Morbid Anatomy of a Case of Hereditary Ataxia," *Brain*, London, Vol. XX (1897), pp. 276-88.



part of the fasciculus gracilis, and to a less extent the posterior part of this fasciculus and the most medial part of the fasciculus cuneatus.

In general, then, the lesions in the three cases thus far studied are nearly identical as regards the neurones involved, though there are differences in the extent of the process quite in accord with the differences in clinical symptoms as the cases were more or less advanced. Dr. Meyer interprets the degeneration in the posterior funiculus as a degeneration of the collaterals which connect the lower levels with the higher (cerebral and cerebellar) apparatus, comparable to the degeneration of the lateral pyramidal tracts of the lower segments of the cord where the basilar part of the cerebral peduncle and the pyramids of the medulla show no degeneration. I am inclined to look upon the degeneration in the posterior funiculus rather as systemic; *i. e.*, as involving certain only of the stem-fibers and collaterals of the intramedullary continuation of the posterior roots, namely, those corresponding to one period of medullation in the embryo. Recent studies make it appear probable (1) that the medullation of the fibers of the posterior roots corresponds more or less closely to the medullation of their intrafunicular continuations, and (2) that the fibers of the posterior funiculi which terminate in the nucleus dorsalis are terminals of the main ascending limb of bifurcation rather than collaterals. In the near future the writer hopes, in another paper, to enter into a rather full discussion of the relation of the lesions found in these cases to the clinical symptoms presented by the patients during life, a discussion which necessarily involves some consideration of the whole question of co-ordination. The discussion of the relations of the disease to Friedreich's ataxia and similar maladies, and of the validity of Marie's' classification of hereditary cerebellar ataxia is also deferred. It will be of especial interest to contrast the Haud family studied clinically by Klippel and Durante and anatomically by Thomas and Roux with the cases described above. In the French family the lesion involved chiefly the ventrolateral cerebellar fasciculus of Gowers'; Meyer's studies and my own show that, in the American family described by Sanger Brown, the dorsolateral cerebellar tract is the one which degenerates. The present report together with that of Dr. Adolf Meyer, makes it very clear that the morbid anatomy of the affected members of the family described by Dr. Sanger Brown presents very constant features; we now know with certainty the neurone-systems principally involved in the individuals who are affected, though we are as yet entirely ignorant as to why just these neurone-systems should be picked out.

#### LEGENDS FOR FIGURES (PLATES XXVIII-XXXIX)

FIG. 1.—Case XVIII. Cerebellum. Facies superior.

FIG. 2.—Case XVIII. Cerebellum and rhombencephalon. Facies inferior.

FIG. 3.—Case XVIII. Cerebrum. Hemisphærium dextrum, polus frontalis.

FIG. 4.—Case XVIII. Cerebrum. Hemisphærium sinistrum, polus frontalis.

FIG. 5.—Case XVIII. Cerebrum. Hemisphærium sinistrum, polus occipitalis.

FIG. 6.—Case XVIII. Cerebrum. Hemisphærium dextrum, polus occipitalis.

\* P. MARIE, "Sur l'héréditaire ataxie cérébelleuse," *Semaine méd.*, Paris, Vol. XIII (1893), pp. 444-7.

- FIG. 7.—Case XVIII. Cerebrum. Hemisphaerium dextrum, facies convexa.  
 FIG. 8.—Case XVIII. Cerebrum. Hemisphaerium sinistrum, facies convexa.  
 FIG. 9.—Case XVIII. Cerebrum. Hemisphaerium sinistrum, facies inferior.  
 FIG. 10.—Case XVIII. Cerebrum. Hemisphaerium sinistrum, facies superior.  
 FIG. 11.—Case XVIII. Cerebrum. Hemisphaerium dextrum, facies superior.  
 FIG. 12.—Case XX. Cerebrum. Hemisphaerium dextrum, facies convexa.  
 FIG. 13.—Case XX. Cerebrum. Hemisphaerium sinistrum, facies convexa.  
 FIG. 14.—Case XX. Cerebrum. Hemisphaerium dextrum, facies medialis.  
 FIG. 15.—Case XX. Cerebrum. Hemisphaerium sinistrum, facies medialis.  
 FIG. 16.—Case XX. Cerebrum. Facies superior.  
 FIG. 17.—Case XX. Basis cerebri.  
 FIG. 18.—Case XX. Cerebrum. Hemisphaerium dextrum, polus frontalis.  
 FIG. 19.—Case XX. Cerebrum. Hemisphaerium sinistrum, polus frontalis.  
 FIG. 20.—Case XX. Rhombencephalon and cerebellum. Ventral view.  
 FIG. 21.—Case XX. Cerebellum. Facies superior. Left half.  
 FIG. 22.—Case XX. Cerebellum. Facies superior. Right half.  
 FIG. 23.—Case XX. Cerebellum. Facies inferior. Right half.  
 FIG. 24.—Case XX. Cerebellum. Facies inferior. Left half.  
 FIG. 25.—Case XVIII. Cerebellum. Sagittal section. Near median plane of vermis.  
 FIG. 26.—Case XX. Cerebellum. Sagittal section. A little lateral from median plane of vermis in front, but passing somewhat obliquely so as to cut through most medial part of the hemisphere behind.  
 FIG. 27.—Section through the cerebellar hemisphere of a man dead of myasthenia gravis; supposedly normal distribution of Purkinje cells.  
 FIG. 28.—Section through cerebellar hemisphere of Case XX; same magnification as in Fig. 27.  
 FIG. 29.—Section through vermis superior of Case XX; same magnification as in Fig. 27.  
 FIG. 30.—Section through a portion of the nucleus dentatus cerebelli of a presumably normal case.  
 FIG. 31.—Section through the nucleus dentatus cerebelli of Case XX, showing diminution in number of cell-bodies. The diminution is more pronounced in this section than in many other sections, but there are numerous places where the cells are as few as here represented.  
 FIG. 32.—Section through a portion of the nucleus olivaris inferior, showing the number of cells in a presumably normal case.  
 FIG. 33.—Section through a portion of the nucleus olivaris inferior of Case XX. The section is thinner than that of Fig. 32, so that the decrease in the number of cells is not so great as might at first sight appear. There is moderate diminution, however, throughout.  
 FIG. 34.—Section through the nucleus dorsalis [Clarkii] of the lower thoracic cord from a case of myasthenia gravis. Number of cells presumably normal.  
 FIG. 35.—Section through the nucleus dorsalis [Clarkii] of the lower thoracic cord of Case XX, showing remarkable loss of cell-bodies.  
 FIG. 36.—Section through colliculus inferior, and pars basilaris pontis of Case XX. Weigert preparation.  
 FIG. 37.—Section through pons at level of brachium pontis, Case XX. Weigert preparation. The brachium conjunctivum shows some thinning of its fibers under the microscope, though this is not brought out in the illustration.  
 FIG. 38.—Section through the upper part of the medulla oblongata, Case XX.

FIG. 39.—Section through the medulla oblongata at the level of the lower part of the nucleus olivaris inferior, Case XX.

FIG. 40.—Section through the medulla oblongata at the upper part of the decussatio lemniscorum and nucleus olivaris accessorius, Case XX.

FIG. 41.—Section through the lower part of the medulla oblongata at the level of the decussatio pyramidum, Case XX. The degeneration of the direct cerebellar tracts is well shown. The degenerated area on one side contains islands of medullated fibers, probably dislocated fibers of the lateral pyramidal tract.

FIG. 42.—Section through the cervical enlargement of the spinal cord of Case XVIII.

FIG. 43.—Section through the spinal cord of Case XVIII. Pars thoracalis.

FIG. 44.—Section through the spinal cord of Case XVIII. Intumescentia lumbalis. Level of T xii.

FIG. 45.—Section through the spinal cord of Case XX. Pars thoracalis.

FIG. 46.—Section through the spinal cord of Case XX. Intumescentia lumbalis. Level of L iii.

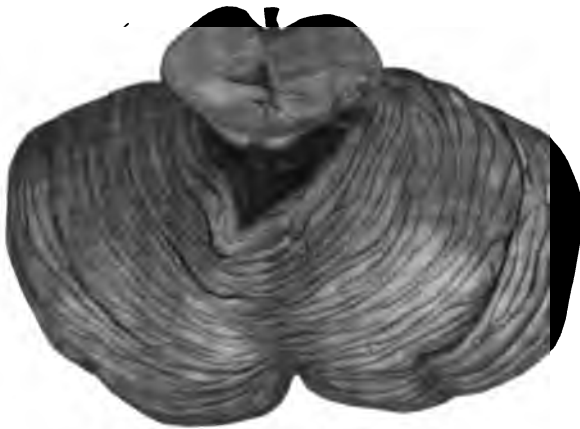


FIG. 1



FIG. 2



FIG. 3

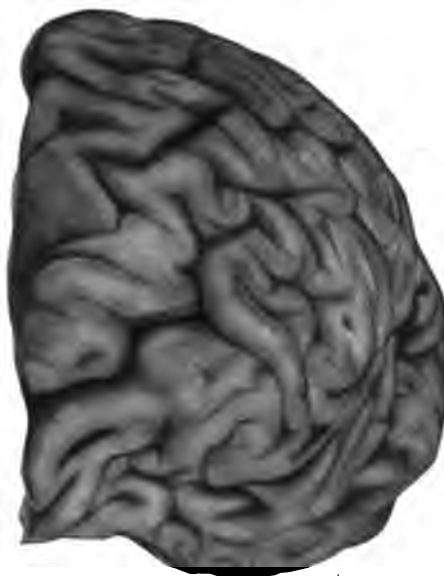


FIG. 4



FIG. 5



FIG. 6





FIG. 7

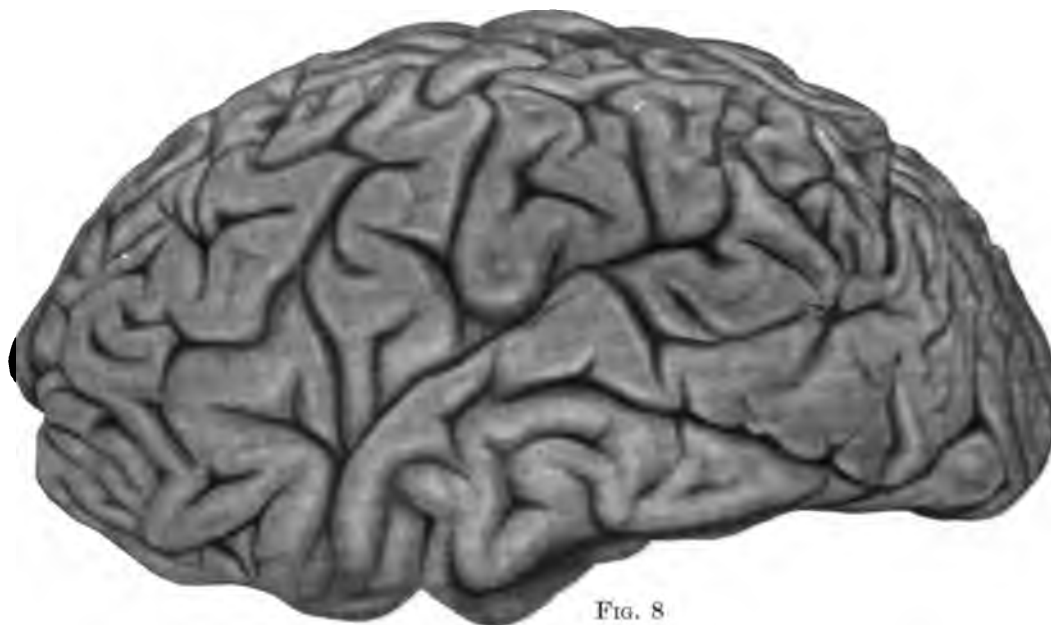


FIG. 8

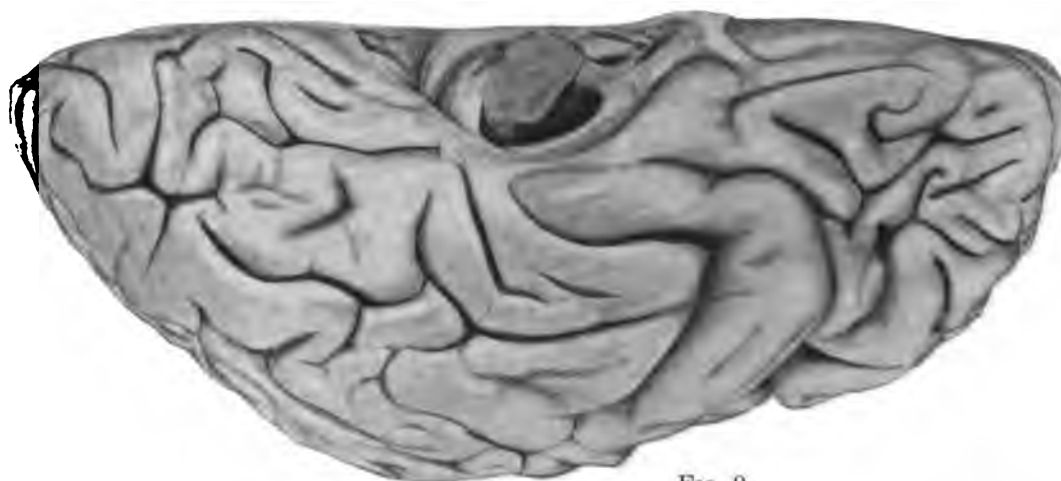


FIG. 9





FIG. 10



FIG. 11



FIG. 12







FIG. 13



FIG. 14



FIG. 15





FIG. 16



FIG. 17





FIG. 18



FIG. 19



FIG. 20



FIG. 21



FIG. 22



FIG. 23



FIG. 24



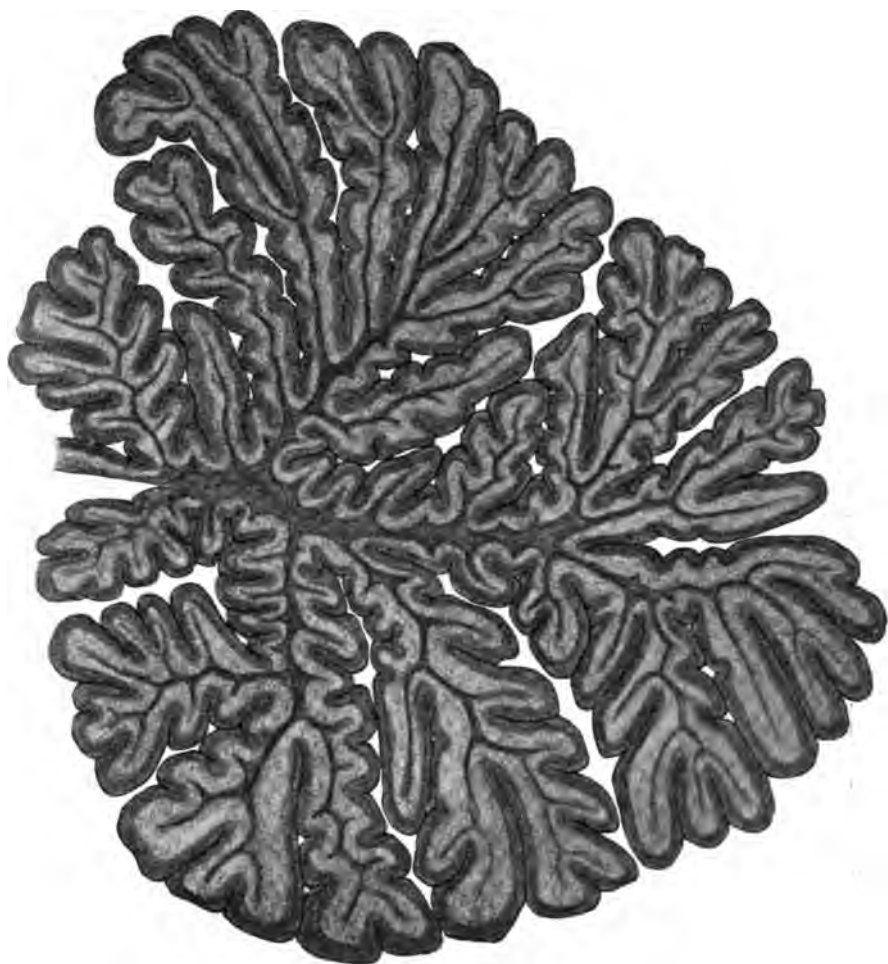


FIG. 25



FIG. 27



FIG. 28



FIG. 29

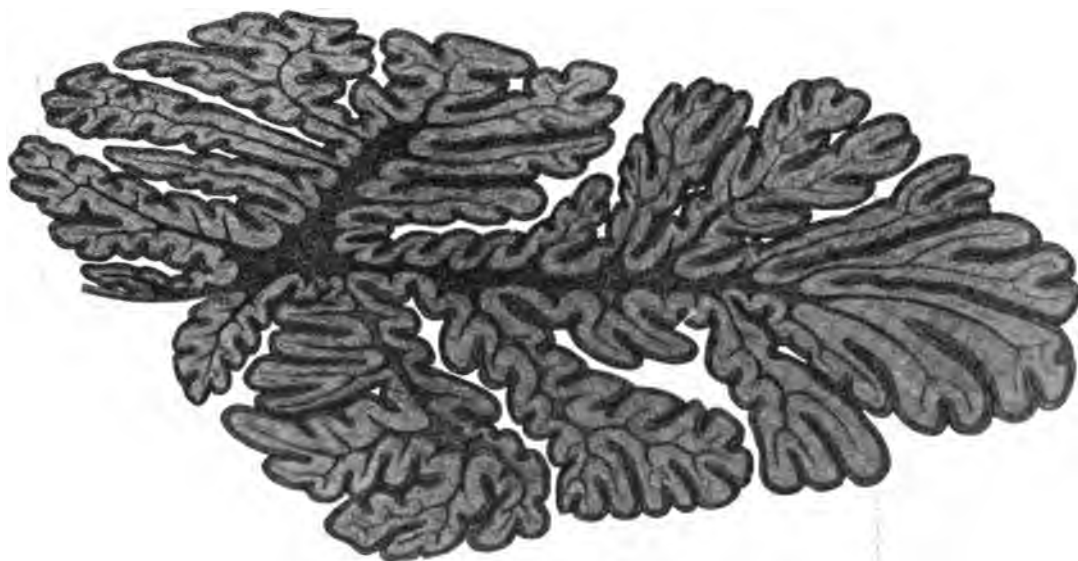


FIG. 26







FIG. 30

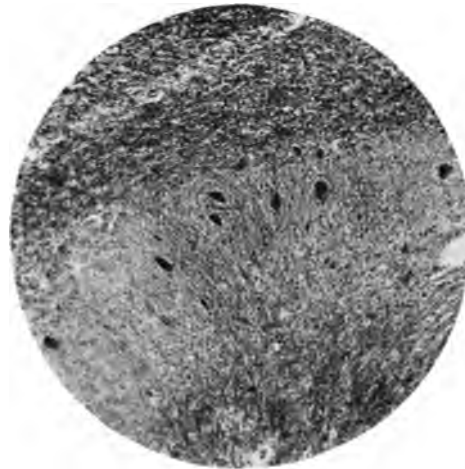


FIG. 31

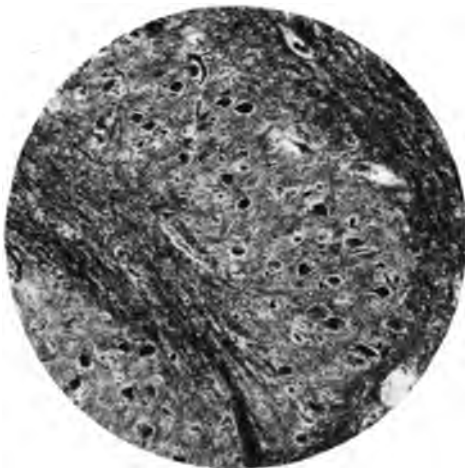


FIG. 32

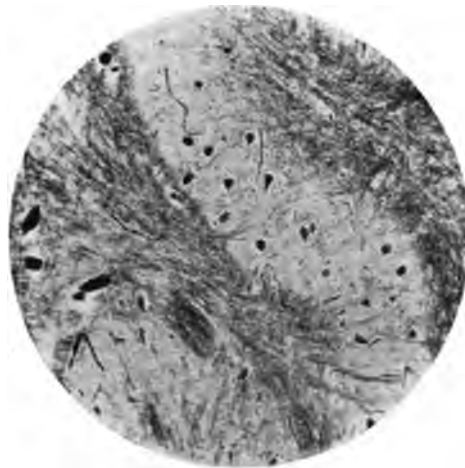


FIG. 33

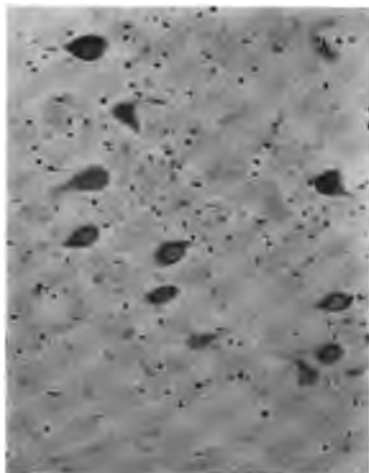


FIG. 34.

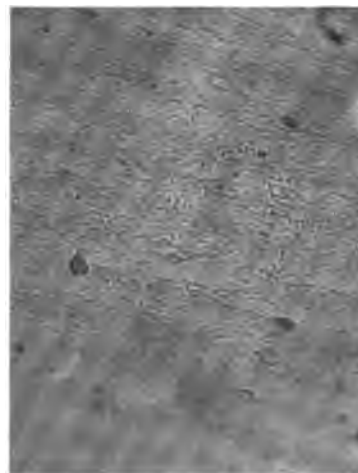


FIG. 35



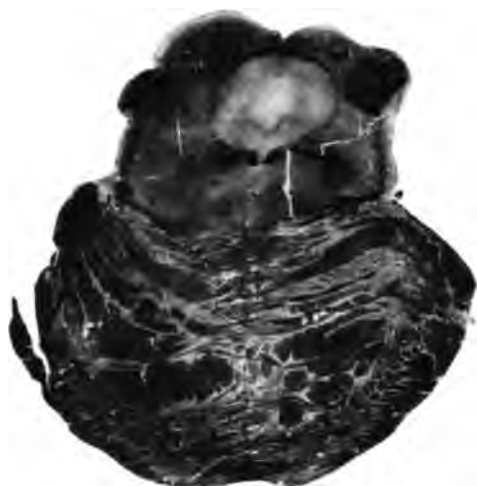


FIG. 36



FIG. 37

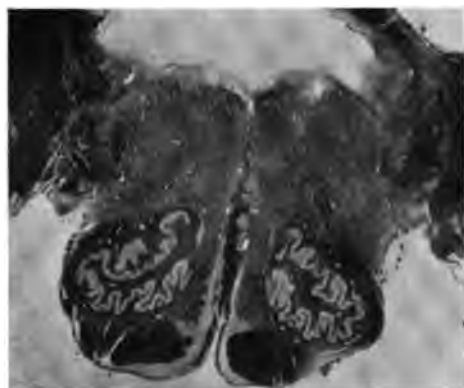


FIG. 38



FIG. 39



FIG. 40



FIG. 41





FIG. 42



